Background

In Spring 2003, Malcolm Burson, the Quality Assurance Officer for the Maine Department of Environmental Protection (MDEP), circulated a letter concerning the quality of data generated by Maine laboratories. The letter was applicable to both commercial laboratories and to municipal and wastewater treatment plant laboratories. Because the MDEP relies on this data to assess environmental conditions and make regulatory decisions they realized that some standard procedures needed to be formalized concerning quality assurance and quality control (QA/QC) in these labs.

Recognizing that commercial laboratories have been using standard procedures and policies for years, the MDEP decided to contract with Katahdin Analytical Services, Inc. (Katahdin) to provide an initial training on QA/QC. The MDEP had funding available through a Supplemental Environmental Program (SEP) with Domtar Industries. The funds were forwarded to the Joint Environmental Training Coordinating Committee (JETCC) who contracted with Katahdin and facilitated the training program on behalf of the MDEP.

The initial manual and training was presented as a first step to introduce wastewater treatment plant personnel to a laboratory QA/QC program. The manual was not in a finalized state at this point. Using input from the training sessions and any input received during the official comments period, the manual was finalized jointly by Katahdin Analytical Services, Inc. and the Maine DEP in October of 2004.

The final version of the manual is provided on CD ROM to facilitate customizing for your facility. The material is solely intended for use by each individual facility. The manual should not be reproduced for other training purposes.

Acknowledgment

This manual is the compilation of a Quality Assurance Manual and a Methods Manual. The Quality Assurance Manual (QAM) was written by Katahdin Analytical Services on behalf of the Maine Department of Environmental Protection (MEDEP). The methods manual was written by the Maine Wastewater Control Association (MWWCA). The QAM is meant to be used as a template for your facility. It was written specifically for wastewater laboratories and not for commercial laboratories. It should be customized, as appropriate, for your needs. The methods manual contains Standard Operating Procedures that may also need to be customized for your facility. Updates to both the QAM and the methods manual templates will be available on the MEDEP and the MWWCA websites. The website addresses are:

MEDEP: www.maine.gov/dep
MWWCA: www.mwwca.org

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The Pennsylvania Department of Environmental Protection (some QAM material & attachments)

FACILITY NAME

QUALITY ASSURANCE MANUAL

DATE

DRAFT

FACILITY ADDRESS

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Maine Wastewater Control Association Laboratory Manual

Introduction
Residue, Non-Filterable (Total Suspended Solids) .....EPA Method 160.2
Settleable Solids .....Std. Method # 2540-F
Temperature.....Std. Method # 2550-B
Total Chlorine Residual DPD Colorimetric .....Std. Method # 4500-Cl-G
pH Analysis .....Std. Method # 4500-H+B
Total Phosphorus .....Hach Method 8190
Biochemical Oxygen Demand (BOD_5).....Std. Method # 5210-B
Escherichia coli Colisure® Procedure.....Std. Method # 9223 and Idexx
Escherichia coli Membrane Filter Procedure.....Std. Method # 9213-D
Fecal Coliform Membrane Filter Procedure .....Std. Method # 9222-D

1.0 INTRODUCTION

1.1 Purpose and Scope

This manual details the quality assurance program in effect at Facility Name. It is meant to be a teaching tool and source of information for laboratory personnel. The Manual is based on Good Laboratory Practices, technical knowledge, industry-accepted standard analytical practices and common sense.

The Manual must be read and understood by all laboratory personnel as part of their training program. The Manual should also be referred to regularly as a source of information. A system of continuous updating is built into the Manual to allow it to change as laboratory conditions change or as new regulations are promulgated. *Terms, definitions and acronyms used throughout this manual can be found as Attachment 1.*

Whenever a technician or analyst is in doubt as to proper procedures in a specific circumstance, the Manual should be consulted. Omissions or errors should be immediately reported to the person responsible for maintaining this manual. **IT IS THE RESPONSIBILITY OF EACH LABORATORY WORKER TO ENSURE THAT THE PROVISIONS OF THIS MANUAL ARE FOLLOWED**. Disagreement with specific requirements or knowledge of changes causing deviation from the procedures should be discussed with the immediate supervisor before further work is completed. Laboratory personnel are encouraged to comment on the Manual and make recommendations for more efficient procedures.

The latest revision of the Manual is the applicable rule. *Check the MEDEP website for any future revisions.*

1.2 Quality Assurance Objectives

Facility Name is committed to the philosophy that quality operations result from quality planning, design, and work performance by skilled operational personnel. Facility Name's policy is to perform its varied types of technical work in accordance with standard quality assurance practices such as those put forth in the Good Laboratory Practices (GLP), various EPA guidance documents, and Standard Methods. A designated staff member shall be responsible for all aspects of the quality assurance program including maintenance of standard operating procedures, laboratory audits, proficiency tests, and quality assurance training documentation.

Objectives of the quality program are:

- to provide representative data of documented quality to regulators
- to promptly identify variances and implement corrective actions
- to maintain readily identifiable and retrievable records that provide documentary evidence of the quality of activities performed

- to maximize the number of valid results by:
    - selecting the appropriate methodology
    - by performing sufficient QC samples to document control
    - by documenting all aspects of the analytical system
    - by adhering to designated holding times
- to ensure that samples collected and tested are representative of the sampled environment through selection of:
    - appropriate sampling protocols
    - proper sample handling procedures
    - appropriate selection of holding times and analytical procedures
    - proper sample preservation
    - prompt extraction and analysis
- to optimize accuracy and precision data through the use of analytical procedures that minimize biases by using:
    - standard procedures
    - traceable standards
    - by calibrating analytical equipment within established acceptance limits
    - by implementing corrective action when measured accuracy and precision exceed pre-established limits
- to maintain traceability so that documentation explicitly describes the history of each sample from collection to analysis

2.0 MONITORING PROGRAM & PERMIT REQUIREMENTS

A wastewater treatment facility laboratory may analyze samples for both compliance with requirements specified by the plant's wastewater permit or for process control. The requirements of a plant's permit vary with the plant's processes and with the classification of the waterway that is affected. No matter which tests are specified, all permittees are required to comply with the regulations set forth in 40 CFR, Part 136 with regard to test methods used. You are responsible to ensure compliance with updates to the 40 CFR, Part 136. Refer to: http://ecfr.gpoaccess.gov .

A tabular format listing the permit required tests, the sample location, the sample type, and the monitoring frequency should be included. *Refer to an example in Attachment 2*. In addition, a tabular format listing process control monitoring tests, the sample locations, the sample types, and the monitoring frequencies should also be included. Refer to an example in Attachment 2.

Typically, a Discharge Monitoring Report (DMR) Form (DEP 49 Form) will have to be completed monthly in accordance with the Maine Pollutant Discharge Elimination System (MEPDES) Permit Program Instructions. The 49 form (*refer to an example in Attachment 3*) is completed from the laboratory bench sheets. The forms are preprinted and coded to include all of the effluent parameters as required by the permit.

3.0 ORGANIZATION, PERSONNEL, & TRAINING

It is important for efficient laboratory operation that all persons responsible for laboratory activities from sampling to preparation and signing of the Discharge Monitoring Report understand the operational structure and specific responsibilities within the organization.

3.1 Laboratory Organization

Include a listing of all individuals involved in laboratory activities ranging from the technicians, assistant operators, Chief Operator, Superintendent and Town Manager. Also include an organizational chart. *Example organization charts are included as Attachment 4.*

3.2 Responsibilities

It is the individual responsibility of all facility staff involved in lab work to perform their assigned tasks according to this QA Manual and to applicable SOPs. This includes responsibility for performing quality control analyses as specified in the method SOP and for entering the QC data in the appropriate logbook, electronic database, or method control file system.

In a well-staffed facility the Superintendent and QA Officer may have the following responsibilities. In a facility with only a few employees, this manual should state who is responsible for the following. **For the purposes of this document we are using the term superintendent, which* may *be customized for your facility. Refer to definitions in Attachment 1.*

The Superintendent shall assure that analysts and technicians are instructed in the requirements of the Laboratory QA Manual and SOPs for the analytical method or other procedure. The Superintendent shall review sample QC data to assure that QC analyses are being performed at the required frequency, that data are documented in the appropriate logbook, electronic database, and that established corrective action procedures for out-of-control situations are followed and the results documented. It is the responsibility of the Superintendent to assure that data have been validated and reported to the appropriate person.

The Quality Assurance Officer (QAO), or designated person (Superintendent), should be responsible for conducting systems audits and inspections for compliance with this manual and laboratory SOPs. This person shall be responsible for maintaining historical files of all QA documents, reviewing QC charts, documenting findings and corrective actions, reviewing training records, managing PTs, maintaining conformance with certification requirements and reporting findings related to all of the above to the Town Manager.

3.3 Training

Appropriately and adequately trained staff is one of the most important elements necessary to meet data quality objectives. An appropriately trained analyst has achieved an

acceptable level of skills and understanding, sufficient to generate data of documented and acceptable precision and accuracy as defined in the applicable method. A trained employee is able to competently carry out the defined duties of his/her job. Competence is based on adequate and sound educational background, specific training to perform assigned duties, and experience in the use of techniques and methodologies employed. A trained employee is able to apply a method and/or technique with sound judgement, demonstrate effective problem solving, perform at an acceptable level of independence, and meet or exceed minimum standards for productivity and data quality. A trained employee understands the basic principles of quality control and quality assurance and their application to the task at hand.

A form should be used to document technical training for each analytical method (*Refer to an example in Attachment 5*).

Training should be conducted in a two-fold manner.

- First, the trainer demonstrates the procedure to the employee.
- Second, the employee conducts the procedure while the trainer observes and discusses the procedure with the employee.
- A training form should be completed for each procedure in which the analyst is being trained.

It is recommended that Initial Demonstration of Proficiency (IDP) and Continued Demonstration of Proficiency (CDP) be documented annually within each employee's training files. This can best be accomplished by the analysis of four known spikes (LCSs) prepared by the analyst, or by the analysis of a known spike (PT or DMR-QA) obtained from an outside source. The recoveries of these spikes must meet the acceptance limits described in the method or SOP.

4.0 QA MANUAL & STANDARD OPERATING PROCEDURES

4.1 QA Manual

This document describes policies related to operation of the analytical laboratories. It provides overall guidance regarding acceptable practices and discusses each element of the Quality Assurance Program. Adherence to the practices described in this manual is required of all employees. This manual may be revised with the written authority of the Superintendent.

4.2 Standard Operating Procedures Manuals

A written SOP manual should contain procedures related to:

- sample collection
- storage
- preparation
- analysis
- disposal
- data validation
- data reporting
- employee training and safety

Each SOP within the manual should contain:

- numbered sections structured in a step-wise manner
- a description of all record-keeping requirements for each step in the SOP
- examples of forms used included as tables or figures and referenced within the text

A designated person shall keep an inventory of SOP numbers, implementation dates and review dates.

For appropriate examples refer to the Maine Wastewater Control Association (MWWCA) SOPs. Be aware that these are under constant revision. *Please refer to the MWWCA website for updates*: www.mwwca.org. Each Standard Operating Procedure should contain at a minimum, the following information:

Title - The name of the concerned task

SOP Number - The internal document control number assigned and tracked by the QA Department

Acceptance - The signature of the originator(s), Quality Assurance Officer and appropriate operations management authority to officially adopt the procedure

Date - Date of issue of most recent revision

Scope and Application - An explanation of the objectives of the procedure, typical applications and limitations

Responsibilities - Identification of the individuals (by title or organizational position) and their responsibilities in performing and facilitating the tasks governed by the SOP

Summary of Method - A short synopsis of the chemistry involved in the procedure.
Interferences - Any factors that may interfere with the proper performance and/or outcome of a procedure and that could compromise the results.

Apparatus and Materials - A complete list of the equipment, apparatus, etc. needed for the procedure

Reagents - A complete list of the reagents, standard solutions, solvents, etc. needed for the performance of this procedure

Sample Collection, Preservation and Handling - Any special considerations needed to assure the integrity of the sample and, consequently, the analytical process.

Method/Procedure - A clear description of the task on a step-by-step basis. The method description should be written clearly enough, and in sufficient detail, to ensure that any two persons performing the procedure will achieve equivalent results. Acceptable and equivalent alternatives should be addressed whenever possible, and described in the same detail. Where applicable to the SOP, the procedure should include a discussion of sample preparation and calibration requirements and also a summary of the automated and manual calculations performed as well as reporting requirements, including data flow charts as appropriate. The SOP should address differences between a published method and the facility's performance of that method, if any exist.

Quality Control Requirements and Acceptance Criteria and Corrective Actions - An outline of quality control requirements, including, procedures, frequency requirements, and acceptance criteria. Corrective actions include a description of what must be done, when and by whom in instances when the QC requirements are not met. This section may be in the form of a table.

Applicable Documents/References - A listing of pertinent, supporting procedure or reference documents such as methods, manuals and/or SOPs

5.0 FACILITIES & GENERAL LABORATORY SAFETY

5.1 General Information

Describe the physical layout of the facility and where the laboratory is located. Provide a floor plan of the facility.

5.2 Laboratory Safety

All applicable standards of the Maine Department of Labor shall apply for municipal facilities and the Occupational Health & Safety Administration for commercial facilities.

It is Facility Name's goal to maintain a safe and healthful work environment. All requirements of the U.S. Occupational Health and Safety Administration (OSHA) and the Maine Department of Labor (MDOL) regarding the equipment and safety procedures in both sample receiving areas and laboratories must be met. Suitable hoods, respirators, protective clothing and eye wear, gloves and/or other measures to prevent or minimize staff contact with hazardous substances must be provided as required. Safety equipment such as eyewash stations, drench showers, fire blankets, spill adsorbents and neutralizers, fire extinguishers, and first aid materials must also be available, if required and should be available even if not required by OSHA or MDOL regulations.

A designated individual at the facility should oversee Environmental Health & Safety aspects. For larger facilities, a safety committee may be formed. The Safety Committee should meet on a regular basis to discuss any new safety or health related issues in the laboratory. The Safety Committee, or designated person, should:

- help to prepare and maintain safety-related SOPs
- conduct an orientation session with each new staff member to familiarize him/her with routine and emergency safety procedures and equipment
- conduct a tour of the laboratory

During the tour the following should be discussed or demonstrated:

- needs for eye, skin, and respiratory protection
- the use of safety glasses, face shields, goggles, partial and full-face respirators which should be issued after respiratory protection training
- the use of ventilated work areas, fume hoods, gloves and Tyvek coveralls
- the location and use of eye wash stations, drench showers, fire extinguishers, and first aid equipment
- requirements for fire and spill notification, emergency procedures, and evacuation

Employees shall be responsible for their own safety. Managers may require that certain levels of protective equipment be worn, when in their judgment, it is appropriate.

The following are basic safety tips to follow when working in a laboratory area:

5.2.1 Always use proper safety goggles or a face shield when performing any test where there is potential danger to the eyes.

5.2.2 Use care when making rubber-to-glass connections. Gloves should be worn when making such connections to prevent injury in the case of breakage. Use a lubricant such as water or glycerin, but never grease or oil. Never force the rubber stopper into the glass tubing. Hold the glass tubing as close to the end being inserted as possible to prevent breaking.

5.2.3 Always check labels on bottles carefully to make sure that the proper chemical is being selected. Keep storage areas clean and organized. Never store incompatible chemicals together, i.e. acids and bases or oxidizers and flammables.

5.2.4 Never handle chemicals with bare hands. Always wear appropriate gloves when handling dry chemicals.

5.2.5 Always work in a fume hood with appropriate ventilation when using chemicals or samples with toxic fumes.

5.2.6 Never take food or drinks into the laboratory area or chemicals or samples into areas designated for eating.

5.2.7 Use care when handling hot equipment or glassware.

5.2.8 Never smoke in the laboratory area.

5.2.9 Never pipette by mouth. Always use appropriate pipettes and bulbs.

5.2.10 Always be aware of the relevant safety information on the chemicals and reagents used in your work area. The Material Safety Data Sheets (MSDS) provide information concerning the safe handling of chemicals, their storage, hazards, first aid and disposal. An MSDS should be available from the manufacturer for every chemical used in the laboratory. These should be easily accessible to all employees.

5.2.11 Do not use damaged or broken glassware. These should be disposed of in a separate container for broken glassware.

5.2.12 Always add acid to water first unless the procedure specifically requires the reverse. If water must be added to an acid or a base first, do so very slowly in a fume hood, stirring the solution as the water is added. Note that this reaction may produce significant heat.

5.2.13 Always wear appropriate personal protective equipment (PPE) such as a lab coat, goggles, safety glasses, gloves, etc.

5.2.14 Be sure that an eyewash station and a shower are available in case of a chemical spill. Immediately rinse affected area with large amounts of water.

5.2.15 Be sure that personnel are trained in the use of fire extinguishers.

5.3 Security

Provide a description of the facility's security system and access to the facility by employees and visitors.

6.0 MATERIALS, APPARATUS & EQUIPMENT

A wastewater laboratory must have all of the necesssary equipment to perform the required tests, to prepare the required solutions and standards and to perform the required quality control samples. Evaluation and selection of suppliers and vendors is done primarily on the basis of the quality of their products, their ability to meet the demand for their products on a continuous and short term basis, the overall quality of their services, their past history, and competitive pricing. This is accomplished through evaluation of supporting documentation provided by the vendor such as certificates of analysis and recommendations. All consumables and equipment must be inspected to assure that they conform to the specified quality requirements of the methods.

6.1 Stock Standards, Reagents, Solvents & Media

6.1.1 Selecting a Grade

To ensure that all chemical standards, reagents, solvents, and media conform to specified quality requirements, only reputable chemical suppliers are used in the laboratory. When in doubt, most standards, reagents, solvents, and media come with specifications concerning their purity. These specifications should be checked to verify that the purity of the standards, reagents, solvents, or media meets the needs of the particular method. The manufacturers can also be contacted to determine if there are specifications for the analytes of interest.

6.1.2 Inspection & Documenting Date of Receipt

Upon receipt, chemical standards, reagents, solvents, and media are checked for possible damage incurred during shipment. If the standards, reagents, solvents, and media are found to be intact, the bottles are then labeled with the date of receipt and the letters "DR" to indicate "date of receipt." The purpose of documenting date of receipt on the bottles/cartons is to facilitate rotation of the laboratory inventory so that the oldest (first received) products are utilized first.

6.1.3 Documenting Expiration Date

The expiration date listed on the standard, reagent, solvent, or media container label should be noted and circled. If no expiration date appears on the label, it is recommended that an expiration date of 5 years for reagents and one year for standards from the date of receipt be assigned. The bottle is then labeled with the assigned expiration date and the letters "EXP" to indicate "date of expiration." This assigned date shall be the effective expiration date unless it is shown through analysis that the standard, reagent, solvent, or media has degraded or become contaminated. The material must be discarded if this is the case.

The purpose of documenting date of expiration on the container is to prevent the use of chemical standards, reagents, solvents, or media that have exceeded their practical shelf life. Some methods may require shorter expiration dates or restandardization to verify the concentration and/or purity.

6.1.4 Storage

Chemical standards, reagents, solvents, or media labeled with the dates of receipt and expiration are then stored in the assigned storage location in the appropriate laboratory area. Commercially prepared standards, reagents, solvents, and media must always be stored according to the manufacturer's directions. Some materials may require refrigeration or freezing. Those that are sensitive to the light should be stored in dark bottles and in a cool, dark place. Be sure not to store incompatible chemicals together.

6.1.5 Documenting Date Opened

When a new (unopened) standard, reagent, solvent, or media is needed for use in the laboratory the oldest (first received) bottle is opened. The chemist who opens the bottle immediately labels it with the date opened and the letters "DO" to indicate, "Date opened."

6.1.6 Traceability

Whenever a chemical standard, reagent, solvent, or media is used for an analysis, the manufacturer's lot number must be recorded on the raw data or appropriate logbook page. This is required to ensure the traceability of the reagents, solvents, and media used for an analysis. This is especially important as a corrective action tool when contamination of a reagent, solvent, or media is suspected.

6.1.7 When the practical shelf life of a chemical standard, reagent or solvent has expired the material is disposed of in accordance with the appropriate disposal procedures.

Waste generated in the laboratory is applicable to both federal and state hazardous waste management regulations:

U.S. Environmental Protection Agency Regulations – 40 CFR Parts 260-299
Maine Department of Environmental Protection Rules – 06-096, Chapters 850-857

6.1.8 Preparation and Dilution

If a preparation or dilution of a chemical standard, reagent, solvent, or media is required, a similar documentation procedure to that listed above shall be used. Instead of recording the date received, the date prepared shall be recorded with the preparer's initials. A date of expiration shall be assigned keeping in mind the expiration date of the native reagent or solvent. The expiration date of the solution or dilution shall not exceed that of the native reagent or solvent. The expiration date assigned to dilutions shall be no more than one year, depending on the analysis. Please refer to individual method SOPs for more detail on reagent and solvent shelf lives. In addition, the stock manufacturers' lot numbers must be recorded on the container.

6.1.9 Lot Numbers

As with stock reagents/solvents/media, the lot numbers of all dilutions and solutions should be recorded on the raw data or appropriate logbook page.

6.1.10 In addition to labeling of bottles, receipt and preparation activities should be recorded in a logbook. This will help to maintain traceability and to ensure that standards, reagents, solvents and media are always prepared consistently. *Refer to an example label, standard documentation requirements and a sample logbook page in Attachment 6.*

6.2 Laboratory Equipment

6.2.1 Refrigerator/Freezer Temperature Logs

Refrigerators and freezers used for sample and materials storage are checked every weekday to ensure that they are operating properly and within established temperature ranges. Refer to section 6.6.2 for thermometer information. All information is recorded in logbooks or on benchsheets *Refer to an example in Attachment 7*. Routine maintenance such as defrosting is performed as needed.

6.2.2 Incubators

Incubators are required for the Biochemical Oxygen Demand (BOD) test and for coliform testing. Incubators must be capable of maintaining the required temperature. The temperature shall be monitored every day of use to ensure that they are operating properly and within established temperature ranges. Refer to section 6.6.2 for thermometer information. All information is recorded in logbooks or on benchsheets.

6.2.3 Desiccators

Desiccators are containers that are used to provide a moisture-free environment to cool objects and chemicals. Desiccators are used in gravimetric tests to prevent samples from trapping moisture. The container must have an airtight seal. The bottom section of the desiccator contains a chemical, or desiccant that absorbs moisture from the air. The desiccant should be color changing so that when it no longer can absorb moisture, the color will change. At this time, the desiccant must be replaced with a fresh supply. To save money, a plastic storage container with a tight cover may be used as a desiccator. A metal trivet can be placed inside to keep the contents from coming into contact with the desiccant.

6.3 Glassware

All glassware used in the laboratory must be maintained in good condition, cleaned, properly stored, and separated according to its specific laboratory application. Cracked, excessively chipped or otherwise defective glassware must be discarded or repaired. All method references give example types of glassware to use. All volumetric glassware utilized shall be sufficient to meet the quality control requirements of the method, i.e. to meet the precision and accuracy of the method.

6.4 Glassware Cleaning

Labware (e.g., glass beakers, plastic test tubes, Teflon stirring bars) must be thoroughly and scrupulously cleaned prior to utilization as part of producing analytical data of consistently high quality. In general, after each use, glassware should be washed with soap and water, rinsed with tap water and then rinsed with reagent grade or deionized water. Glassware should be allowed to dry and stored on clean areas as free from dust as possible. Specific glassware procedures depend on the analysis to be performed. Glassware used for the total phosphorus test must be cleaned with non-phosphate detergent and acid rinsed. BOD glassware may be soaked in a bleach solution after cleaning and then rinsed to eliminate any bacterial contamination. These specific procedures should be included in the specific method SOPs.

6.5 Sample Containers

Samples shall be collected in containers as specified by the SOPs. Ideally, all sample containers should be purchased pre-cleaned from a reputable commercial source. In some cases pre-cleaned containers are received with certificates of analysis documenting the concentration levels of applicable analytes for each container type and lot. Certificates of analysis accompanying container lots should be maintained by the laboratory. Alternatively, sample bottles may be re-used if appropriately cleaned.

6.6 Instruments

Laboratory instrumentation used shall be as specified in the protocol for the analytical method. A master list of the major analytical instrumentation currently in use in by the facility should be maintained.

Preventive maintenance is performed for each instrument by manufacturers, analysts and field service technicians on an ongoing basis and the activities documented in a bound instrument maintenance logbook or in the instrument runlogs.

Corrective maintenance shall be provided as required for all instruments and equipment and documented in appropriate logbooks. Factory replacement parts, trained service technicians and first quality materials should be used whenever necessary.

6.6.1 Analytical Balances

Annually, calibration of the entire analytical range shall be checked by a qualified service technician. However, balance calibration must be verified each day of use. Working class weights are used for daily verification. These weights should also be verified against NIST Class I weights on an annual basis. An outside service may be used for this if the laboratory does not have NIST Class I weights available. *Refer to Attachment 8 for guidance on balance verification*.

When developing balance verification criteria, use acceptance criteria that is appropriate for the balance/method. For example, methods that incorporate the use of a four-place balance (TSS) should have acceptance criteria that is

expressed to four places. Some examples of acceptance criteria are given below:

Weight(gm)True Value	Acceptance Criteria (gm)
0.1000	± 0.0005
1.0000	± 0.0005
10.0000	± 0.0005
100.0000	± 0.0005
0.50	± 0.05
5.00	± 0.05
20.00	± 0.10
50.00	± 0.10

6.6.2 Thermometers

Working thermometers are used in the lab to track storage temperatures and to perform methods at required temperatures. The type of thermometer used will be dependent on the method specifications. For example, E-coli samples are incubated at 44.5 °C ± 0.2 °C. A thermometer that is capable of accurately reading ± 0.2 °C must be used. Vendor catalogs usually give a description of the accuracy of each thermometer. Mercury thermometers should be avoided, if possible, due to their hazardous nature. Alcohol or digital thermometers should be used instead.

Each working thermometer should be individually numbered and tagged with an identification number. All working thermometers should be compared with the reference thermometers on, at least, an annual basis. *Refer to Attachment 9 for guidance on thermometer verification.* In addition, working thermometers should be visually inspected by laboratory personnel prior to use. Calibration temperatures and acceptance criteria are based upon the working range of the thermometer and the accuracy required for its use.

Certified, or reference, thermometers are maintained for checking the calibration of working thermometers used during testing. Reference thermometers are provided with NIST traceability for initial calibration and should be recertified every five years, by an outside service, with equipment directly traceable to the NIST.

6.6.3 pH/Electrometers

These meters are calibrated using buffer solutions before use each day, and once after each four hours of use. *Refer to the MWWCA SOP or to Standard Methods for pH meter calibration.*

6.6.4 Spectrophotometers

During use, spectrophotometer performance is checked against initial calibration verification standards (ICVs) and continuing calibration verification standards (CCVs). The instrument operating capability and wavelength verification are also evaluated every year by an outside service.

6.6.5 Ovens

Oven temperatures may be monitored using a thermometer intended for oven monitoring that shall be compared to a NIST traceable thermometer annually. Oven temperature must be checked every day of use and recorded in the appropriate analytical logbook.

6.7 Autoclave

The autoclave is used in the sterilization of equipment prior to bacteriological testing. The autoclave must be capable of developing and maintaining 15 psi at 121 °C for at least 20 minutes. Temperature must be recorded in an autoclave temperature log.

6.8 Pipettes

Pipettes are specially calibrated glass tubes used for accurately transferring small volumes of solution (usually less than 50 mL). Volumetric pipettes are designed for the accurate transfer of a specific amount of liquid. These pipettes have narrow tips with a bulb-like expansion in the middle. The calibration mark is found above the center expansion. These pipettes, typically indicated as Class A, are designed to free-flow until a small amount of liquid remains in the tip.

Measuring pipettes are graduated to deliver varying volumes. These are less accurate. An automatic pipette may be used to deliver varying volumes of liquid more accurately than a measuring pipette. However, these pipettes do require daily calibration if used. They should be inspected before use. All automatic adjustable pipettes must be calibrated each day of use at the maximum volume. If you are interested in checking the quality of pipettes, this may be done so by comparing the true weight of a delivered volume of reagent grade or DI water and weighing. Note that 1 mL of water = 1 gm of water. All recordings should be recorded in a logbook (*refer to an example in Attachment 10.)*

6.9 Reagent Water or Deionized (DI) Water

The performance of analytical work requires that the water used for preparation of samples and reagent solutions, and final rinsing of glassware be “theoretically pure,” i.e. free from interferences, electrolytes, and other contaminants. In all cases, unless specified by the analytical SOP, reagent grade or deionized water is used.

The following tests should be performed to document water quality. The results should be filed.

General Lab Water		
Parameter	**Limit**	**Monitoring Frequency**
Conductivity	<2 umhos/cm @ 25 °C	Monthly

Water for Microbiological Analyses		
Parameter	**Limit**	**Monitoring Frequency**
Conductivity	<2 umhos/cm @ 25 °C	Monthly
Total Residual Chlorine	<detection limit	Monthly
Heavy Metals (Cd, Cr, Cu, Ni, Pb, Zn)	<0.05 mg/L	Annually
Heterotrophic Plate Count	<500 CFU/ml	Monthly

These water quality tests are not mandatory as long as the method blanks performed in accordance with Table 11-1 are free from contamination of the analytes of concern.

7.0 BASIC LABORATORY SKILLS

In order for a laboratory test to produce accurate and representative results, it is critical for analysts to understand the importance of basic laboratory techniques such as measuring, diluting, weighing and calibrating.

7.1 Measuring

Deciding how to measure a volume of liquid depends on several factors – the type of liquid, the amount of liquid and how accurate the measurement has to be. Thick liquids are usually measured best using wide mouth glassware. The larger the volume of sample measured, the less accurate that it needs to be. Typically beakers and flasks are used for measuring larger volumes of liquid and are less accurate. Burettes and pipettes are used for measuring smaller volumes of liquids and are more accurate. Measuring devices will either be "TC" (to contain) or "TD (to deliver).

"TC" glassware is calibrated so that when it is filled to the calibration mark or a certain graduation mark, the liquid column will contain a specific volume of liquid. In dispensing this specific volume, the entire contents of the column must be transferred. A full volume "TC" pipette may require that you "blow out" the last drops of liquid, using a pipette bulb, to deliver the measured volume.

"TD" glassware is calibrated so that when it is filled to the calibration mark or a certain graduation mark, the volume indicated will be delivered upon dispensing the liquid. Full delivery "TD" pipettes calibrated from the zero mark to the pipette tip. After draining the pipette, a few drops of liquid may remain in the tip. This liquid is released by touching the tip of the pipette against the side of the transfer container until no more liquid drains out.

All measuring devices used for liquid volumes have a downward curve at the surface of the liquid. The curved surface is called the meniscus. All measurements taken should be made on the graduation closest to the lowest point of the meniscus. *Refer to Attachment 11 for example glassware and meniscus.*

7.1.1 Graduated Cylinders

Generally, graduated cylinders are less accurate and should only be used for measuring liquid volumes greater than 25 mL. Graduated cylinders should not be used to prepare standard solutions. To use a graduated cylinder, shake or stir the liquid to be transferred to be sure that any solids are mixed. Quickly transfer the liquid to the graduated cylinder before the solids have a chance to settle out. Measure to the meniscus at the graduation mark and then transfer the sample to its final container.

7.1.2 Pipettes

Pipettes are generally more accurate and can be used for liquid volumes less than 25 mL. The liquid should be drawn into the pipette using a pipette bulb, past the zero mark, drained back down to the zero mark, and then the desired

volume of liquid drained into the test container. The liquid being transferred should be stirred or shaken to ensure a homogenous sample.

7.1.3 Burettes

Burettes are typically used for titrations in which an accurate volume of a titrant (known concentration) is dispensed into a known volume of sample (unknown concentration). As titrant is added, a chemical reaction is initiated and proceeds as more titrant is added to a recognizable endpoint (color change, meter reading). At this point, the titration is complete.

A burette is calibrated to be read from the top down. For example, a 25 mL burette will have the zero mark at the top and the 25 mL mark at the bottom, above the stopcock, with many graduations in between. The volume dispensed is read from the lowest point of the meniscus. It is always best to fill the burette past the zero mark and then to empty it so that the zero mark is at the bottom of the meniscus. The burette should be refilled if there are any air bubbles present.

The stopcock at the bottom of the burette is designed to control the flow of liquid. When the lever is horizontal, the stopcock is closed. It is fully open when it is vertical. The burette tip must not be used if damaged, cracked or dirty. Burettes should be acid cleaned periodically.

7.2 Weighing

The type of balance used will depend on the accuracy of the measurement needed. Depending on the required weight, the balance should always be at least one decimal place further than that. For example, if a method says to weigh 5.2 grams, the balance used should be able to see out to 0.01 grams or better. Always refer to the manufacturer's instructions before operating a balance. Also, refer to section 6.0 for additional weighing information.

7.3 Dilutions

When performing analytical tests, it is often necessary to dilute, or to reduce the strength, of a sample or solution. A sample or solution can be diluted by adding a known amount of water to a known amount of the sample or solution. The amount of the dilution will depend on the original strength of the solution or sample and the desired final strength needed for the test.

When dilutions are performed, a dilution factor must be taken into account. The dilution factor is the final total volume divided by the volume of sample or solution used. The result obtained on a diluted sample must be multiplied by the dilution factor to obtain the correct final result.

Example: 20 mL of a sample is diluted with 80 mL of deionized water for a final volume of 100 mL.

100 mL / 20 mL = 5 (dilution factor)

The analytical result is 3.7.

3.7 X 5 = 18.5 final result

When solutions or standards need to be diluted to obtain weaker strengths, the following equation should be used to determine how much to dilute.

$$(V_i)(C_i) = (V_f)(C_f)$$

Where: V_i = Initial volume of original solution/standard
C_i = Initial concentration of original solution/standard
V_f = Final desired volume of diluted solution/standard
C_f = Final desired concentration of diluted solution/standard

Typically, C_i, V_f, and C_f are known values. The analyst needs to determine the original volume (V_i) of a known strength to be added to a specific volume of a desired strength.

Example: You want to make 100 mL of a 5 mg/L chloride solution from a 1000 mg/L chloride standard.

$$(V_i)(C_i) = (V_f)(C_f)$$

$$(V_i)(1000) = (100)(5) \qquad V_i = 0.5 \text{ mL}$$

V_i = Initial volume of original solution/standard
C_i = 1000 mg/L
V_f = 100 mL
C_f = 5 mg/L

In other words, add 0.5 mL of the 1000 mg/L standard to a final volume of 100 mL to make a 5 mg/L dilution.

The following rules apply when making dilutions:

- Samples that are over the calibration or working range of the instrument or analysis must be diluted and rerun.
- Such dilutions should be made to attempt to bring the sample concentration around the mid-point of the standardization.
- Sample dilutions should be performed using volumetric glassware (volumetric pipettes, burettes, or volumetric flasks), calibrated adjustable pipettes or, if available, instrument auto-samplers.
- Sample dilutions should be made so as to maximize the amount of native sample used. This will provide for a more representative portion of sample.

- If a large dilution is required it is better to do a serial dilution rather than using a non-representative aliquot of sample. (Example: A sample requires a 1/1000 dilution. Make a 1/100 dilution of the native sample and then a 1/10 dilution of the initial dilution).

- Dilution factors should be clearly marked on the raw data. It is preferred that the dilution factor is expressed in such a fashion that it may be recalculated, i.e. 1:10 or 1/10 is preferred over 10x.

- When possible, native samples should be poured out of their original containers into small sample cups, before making dilutions, so as not to contaminate the native sample.

- Used pipette tips or other measuring devices should never be inserted into original sample containers.

7.4 Analytical Calibration Procedures

Wet chemistry instruments are standardized for the parameter of interest by the analysis of a set of calibration standards prepared by diluting a stock solution of known concentration. A calibration curve within the working range of the instrument is established by analysis of one to five working standards, including a zero point.

Most instruments in the wastewater laboratory have a linear relationship between response and concentration. The linearity may vary at the high and low ends of the curve where detector saturation or insensitivity come into play. A calibration curve is generated through linear regression with concentration as the x-axis and response as the y-axis. If the instrument is typically adjusted to read zero in the presence of a blank, then the zero point should be included in the linear regression. The calibration must reflect an acceptable correlation of data points or linearity to be acceptable. In cases where the calibration data are outside of these criteria, the analyst must rerun the calibration standards (meeting the same criteria), changing instrumental conditions as necessary until appropriate acceptance limits for the method are achieved. *Refer to Attachment 12 for example calibration curves. Refer to the CD for an example Excel spreadsheet for plotting calibration curves.*

Calibration standards for each parameter are chosen to bracket the expected concentrations of those parameters in the sample, and to operate within the linear response range of the instrument. Sample concentrations that fall above calibration range are diluted and reanalyzed until they are within the calibration range. Calibration standards should be prepared as described in each method or SOP. At a minimum, each calibration should contain at least three standards and a blank. The reporting limit is verified by analysis of a standard at the reporting limit.

An independent standard is analyzed to confirm the calibration. If the calibration is not within acceptance limits, the instrument is recalibrated. The samples are analyzed for the analyte of interest. During sample analysis, a check standard (Continuing Calibration Verification, CCV) is analyzed to monitor instrument stability. If the CCV

indicates that instrument calibration has changed by more than the method specified acceptance limits, the instrument is recalibrated and the analysis is repeated. Following completion of the sample analyses, the check standard is reanalyzed to confirm calibration. If calibration verified, the analysis is completed. However, if the calibration is not verified, appropriate corrective action is taken and effected samples are reanalyzed.

For some analyses that are performed frequently, and for which substantial calibration data are available, a complete recalibration is not required each time an analysis is performed. As long as one calibration standard (Initial Calibration Verification - ICV), analyzed at the beginning of the analysis, does not vary from the expected response (based on the most recent initial calibration curve) by no more than $\pm$25% or as specified by the method, or SOP, whichever is more stringent. If this criterion is not met, a complete recalibration is necessary.

8.0 SAMPLING PROCEDURES

The value of any laboratory analysis performed on a treatment plant sample depends upon the overall quality of the sample on which the test is performed. The sample must be representative of actual conditions in the plant. Often, the error most commonly committed in analytical testing is that of improperly collecting or preserving samples.

The purpose of sample collection is to obtain a portion of the wastewater that is small enough to be conveniently handled in the laboratory and still be representative of the total waste stream. This portion is intended to simulate millions of gallons of flow in some cases. The sample must, therefore, reflect usual conditions for water passing that sample point throughout the day or testing period. The sample must be collected in such a manner that nothing is added or lost in the portion taken and no change, in the analyte(s) of concern, occurs between the time the sample is collected and the laboratory test is performed. Determining the best location for the sample point is critical in providing an appropriate sample (i.e. a sample tap in a dead area of a reservoir or on the floor of a process basin serves little or no purpose in helping the plant operator determine water quality).

8.1 Sampling Techniques and Sample Types

There are two manners in which to take samples…manually and automatically. Manual sampling involves the use of dippers, sample thieves (or weighted bottles) and hand-operated pumps. Automatic sampler units or ISCO's use a computer controlled peristaltic pump to pull samples at regular intervals over a set period of time, thus characterizing flow over time.

Beyond the specifics of the sample matrix and types of compounds to be analyzed, the two classifications of samples that are collected are **Grab Samples** and **Composite Samples**.

8.1.1 Grab samples are samples collected over a period of time not to exceed 15 minutes and they reflect the source material conditions at a particular instant of time. Grab samples are used primarily for analyses that need to be run immediately after collection (i.e. dissolved oxygen, chlorine, pH), but also include those analyses that need the entire contents of a sample container (i.e.Bacteriological)

8.1.2 Composite samples are obtained by taking an appropriate number of grab samples collected at equal intervals or proportional to flow and serve to characterize the average conditions at a sample point over time. Flow proportional sampling takes into account changing sample flow from a volume standpoint. The nature of the composite sample requires that the tested parameters be stable in the composite container for the duration of the sampling, which is often 24 hours and typically involves refrigerating sample to 4^{o} C.

8.2 Sample Containers, Sampling Equipment and Aliquot Size

Using the appropriate sample containers to store the samples prior to analysis is critical to the successful analysis of wastewater samples. The sample container must always be

an inert material that will not contribute to or artificially affect the integrity of the sample. The two major choices available for sample storage are Glass and High Density Polyethylene (HDPE) with Teflon lined caps. A sample taken for metals analysis, for example, is taken and kept in HDPE with the exception of mercury, which should be kept in a glass container since it is susceptible to diffusion through plastic. While the reuse of sampling containers can be an ecologically conscientious choice, this can open up avenues for cross contamination if the bottles are not cleaned properly. Sampling equipment, however, is typically reused and susceptible to the same contamination issues. Use a non-phosphate based detergent and a hot water rinse, acid washing, re-rinse, deionized or reagent grade water rinse and air dry process to ensure cleaning. Use an appropriate acid for the acid wash step (i.e. do not use nitric acid in bottles to be later used for nitrate analysis, or for a sample container to be used for chlorine analysis, do not use hydrochloric) The use of a bottle blank will help to verify the efficacy of the cleaning regiment for reused sample containers.

Allocating the correct sample volume is also a key part of the sampling process. There is a preferred sample size for all analyses that provides just enough sample to achieve low enough detection limits for each method, but not so much sample that then becomes an issue for the laboratory waste disposal.

8.3 Sample Preservation

To ensure representative samples, we do not want the sample characteristics to change during the sampling process, especially while collecting composite samples. Sample preservation is a way to slow down or prevent any change in the sample characteristics. The three processes which act upon samples while in the sample containers are Biological, Chemical, and Physical.

8.3.1 Biological processes result from the microbes that exist in wastewater naturally. As they multiply and grow, they utilize certain nutrients in the water and produce byproducts such as nitrate and acids that can alter the baseline results of the sample. If the sample is being tested for coliforms, the baseline plate counts will change drastically over time.

8.3.2 Chemical interactions are often complex interactions between the many components of wastewater. Often times, sulfides can interact with cyanide to form thiocyanate, removing both chemicals from solution. Nitrite can be oxidized by hexavalent chromium into nitrate changing both concentrations.

8.3.3 Physical processes can involve precipitation and loss of trace metals from solution and volatilization of dissolved gases from liquid and sludge samples.

For on site analysis at the plant, preservation is often not required since the samples are typically run right away, but for work being sent out to an outside lab, the preservation process must be done and done correctly.

8.4 Preservative Systems

The most common preservative steps are:

8.4.1 Cooling the sample to 4^{o} C, which inhibits biological activity, and keeps dissolved gases in solution.

8.4.2 Sodium thiosulfate is used to remove chlorine while copper sulfate, and mercuric chloride is used to control biological growth.

8.4.3 Zinc acetate is used to trap sulfides.

8.4.4 Appropriate pH adjustment is also used frequently (using sodium hydroxide to adjust upwards to over 12 and using hydrochloric, sulfuric or nitric acid to adjust downwards to a pH of less than 2) The appropriate acid is required. You would not want to use nitric acid for a nitrate sample, and likewise, sulfuric acid in a sample being analyzed for sulfates. Acids are critical for keeping target analytes such as metals, in solution. Non-preserved samples must be run immediately.

8.5 Holding Times

There is no topic that is perhaps more critical to the successful operation of any laboratory, than running samples within the accepted holding time for each analysis. The holding time for analytes reflects the allowable time span permitted before the analysis must begin. Due to the sensitive nature or volatility of certain compounds, this permitted time frame within which an analysis must begin is critical to ensuring that target compounds have been verified within a certain margin of accuracy.

On site analysis at the plant is typically done within a few minutes to a few hours of sampling, making the issue of holding times less critical. However, for the work that is sent out for analysis, holding times become a much bigger issue. Analyses like temperature, pH, dissolved oxygen, and total residual chlorine all have an immediate testing requirement, and are either done in the field upon sampling or immediately after receipt in the laboratory. Analyses such as coliform / E. coli shall be run within a 6 hour window to verify bacterial counts (which are forever changing as the ambient bacterial population continue to multiply, sometimes exponentially). A BOD analysis, optimally, should be run within 6 hours of sampling. Whenever a holding time cannot be met, whether the analysis is performed in-house or sent to an outside laboratory, the appropriate DEP inspector should be informed so that a maximum holding time (specific to the particular analysis) can be established.

8.6 Documentation

All activities performed in the field should be documented in a field logbook and on a label to be placed on the sample containers. The documentation in the logbook should include:

- sampling techniques
- conditions such as time, flow rate, etc

The sample label should include:

- time and date of sampling
- the preservative used
- the name of the sampler
- the identification of the sample or site
- the test to be performed on the sample

The following table provides the EPA requirements, found in 40 CFR, Part 136, for some wastewater parameters, for preservation, container, and holding time requirements.

Type of Sample and Holding Time

Parameter	Type of Sample	Holding Time	Sample Container
pH	Grab	Analyze Immediately	Plastic Bottle
Temperature	Grab	Analyze Immediately	Plastic Bottle
Dissolved Oxygen	Grab	Analyze Immediately	BOD Bottle
BOD	Composite Flow Proportional	24 Hours	Composite Sampler Plastic Bottles
Total Coliform Fecal Coliform E. Coli	Grab	Total 24-30 hours Fecal/E. Coli 6 Hour	Sterile Sample Plastic Bottle w/sodium thiosulfate
Chorine Residual	Grab	Analyze Immediately	Opaque BOD Glass Bottle
Total Suspended Solids	Composite Flow Proportional	7 days	Composite Sampler Plastic Bottles
Specific Conductance	Grab	28 Days	Glass
Metals	Grab/Composite	6 Months	Plastic Bottle
Mercury (1631)	Grab	28 days if preserved	Glass
TKN	Grab	28 Days	Plastic Bottle
Settleable Solids	Grab	48 Hours	Plastic Bottle

Preservation Conditions

Parameter	Container	Volume	Preservation	Holding Time	Representative Sampling Time
BOD	P	1 L	4°C	24 Hours	8 AM – 8 AM
TSS	P	1 L	4°C	7 Days	8 AM – 8 AM
TKN	P	.5 L	H_2SO_4 pH < 2.0 4°C	28 Days	8 AM – 8 AM
Oils & Grease	GA	1 L	HCl or H_2SO_4 pH < 2.0	28 Days	Between 8 AM and 12 PM
Metals	P	.2 L	HNO_3 pH < 2.0	6 Months	8 AM – 8 AM
Mercury (1631)	G	0.25 L	BrCl (0.5% v:v)	28 days if preserved	8 AM – 8 AM
Phenols	G	.5 L	H_2SO_4 pH < 2.0 4oC	28 Days	8 AM – 8 AM
Cyanides (T)	P	1 L	NaOH pH > 12.0 4°C	14 Days	2 PM
VOC	V	40 ml	4°C HCl pH <2.0	14 Days	2 PM

G = Glass bottle with Teflon lined lid
GA = Amber bottle with Teflon lined lid
P = Plastic Bottle
V = Approved glass vials with Teflon and pure rubber seals

Note: All samples are refrigerated at 4°C after preservation.

9.0 SAMPLE CUSTODY

Chain-of-Custody encompasses three major elements:

- field sampling
- laboratory analysis
- final data file

A Chain-of-Custody (COC) form documents field activities and laboratory sample handling activities from time of receipt through the analytical process.

Samples may be physical evidence and should be handled according to certain procedural safeguards. All areas of the laboratory in which samples are received, stored, processed, or analyzed shall be kept in a condition that minimizes the risk of samples becoming lost or accidentally destroyed, contaminated, degraded, mis-identified, improperly handled or otherwise compromised.

9.1 Chain-of-Custody

EPA defines evidence of custody in the following manner:

- It is in your actual possession
- It is in your view, after being in your physical possession
- It was in your possession and then you locked or sealed it up to prevent tampering
- It is in a secure area.

Sample custody and sample control procedures ensure that:

- All samples are uniquely identified
- Samples are analyzed as requested and are traceable to their records
- Important sample characteristics are preserved
- Samples are protected from loss or damage
- Any alteration of samples (e.g., filtration, preservation) is documented an
- A record of sample integrity is established for legal purposes

A Chain-of-Custody Form should be completed by field personnel for all samples received by the laboratory. The form should accompany the samples received at the laboratory. The completed Chain-of-Custody Form should include the following information (*refer to Attachment 13 for an example COC form*):

- Facility Name
- Field sample number/identification
- Number and type of containers
- Date and time sampled
- Sample matrix
- Preservative
- Analysis requested

- Sampler signature
- Signature of person relinquishing samples
- Date and time relinquished
- Sampler remarks

The record should be filled out completely and legibly. Errors must be corrected by drawing a single line through and initialing and dating the error. The correct information is then recorded with indelible ink.

9.2 Receipt & Inspection

When samples are received by the laboratory the Chain-of-Custody must be signed by the receiving person. The receiving person, or designated employee, should verify the integrity of samples as they are unpacked. It should be noted whether the samples are received intact or broken, whether the samples are appropriately preserved and properly identified, the temperature of the container, and any other notable observations. This information should be documented on a sample condition form or a sample receipt logbook.

If the integrity requirements are met or after any discrepancies are resolved, the sample is assigned a unique laboratory identification or number and transferred to the appropriate storage location for storage until preparation and analysis. All samples must be stored at 4 °C ± 2 °C, or as specified in the specific method or SOP.

Once samples are in the laboratory, an internal custody record is generated to track the transport and status of each sample from storage to the laboratory and back to storage.

9.3 Subsampling for Sample Preparation or Analysis

In almost all cases, the laboratory receives more sample than is typically used for a specific analytical method. Therefore, a smaller aliquot, or subsample, must be obtained from the container for sample preparation or analysis. Obtaining a representative subsample, i.e. one that has the same characteristics and chemical composition as the original sample, can be difficult without employing complex techniques. In general, the following technique shall be used.

Aqueous Samples

Aqueous samples must be mixed by inverting the sample several times prior to pouring off an aliquot. This inversion must be performed for each subsequent test requiring an aliquot.

10.0 QUALITY CONTROL

A quality control program is a systematic process that controls the validity of analytical results by measuring the accuracy and precision of each method and matrix, by developing expected control limits, by using these limits to detect errors or out-of-control events, and by requiring corrective action techniques to prevent or minimize the recurrence of these events.

10.1 Method Detection Limits (where required)

Method detection limit (MDL) studies should be performed annually. MDL studies are also performed after any significant procedural or instrument configuration change.

Method detection limits should be determined using replicate spiked reagent grade or DI water samples. A minimum of seven replicates of a sample spiked with the analyte of interest is processed through the entire analytical method. The concentration of the detection limit sample should be between one and five times the anticipated detection limit.

When doing the MDL study, the laboratory must calculate the detection limit as the student's t (n-1, 1-$\propto$ = 0.99) times the standard deviation (n-1) of the replicate spiked sample measurements. *Refer to 40 CFR Part 136, Appendix B for further discussion.* The following are the specific steps taken to calculate an MDL:

10.1.1 A minimum of seven replicate analyses of reagent grade or DI water spiked with the analyte(s) of interest are analyzed by the appropriate analytical method on each instrument. All analytes that may be analyzed by a specific method should be included in the MDL study. All seven replicates need not be analyzed in the same batch. Sets of two or three MDL points may be analyzed as part of several analytical batches that are run on different days. Alternatively, one MDL point can be analyzed with every analytical batch and results compiled as needed (rolling MDL).

10.1.2 The concentration of analyte(s) spiked into the reagent grade or DI water should be equal to or in the same concentration range as the estimated MDL. A spike concentration between 1 and 5 times the estimated MDL is optimal.

10.1.3 Each spiked replicate is processed through all steps of the analytical method including any preparatory procedures and the results are calculated according to the procedures outlined in the given analytical method.

10.1.4 If a calibration curve is required to calculate the measured level of an analyte, the standards used to determine the curve should cover the range normally used for sample analysis. The curve should include a minimum of 3 standards and a blank unless the given analytical method or regulatory program requires a method-specific calibration protocol. The lowest calibration standard should be at a concentration 3-5 times the estimated MDL.

10.1.5 All QC samples required by each applicable method must be analyzed in conjunction with the MDL study. These QC samples may include, but are not necessarily limited to LCSs, method blanks, and calibration verifications/checks. If these criteria are not met then the MDL data may be suspect; the cause of the problem should be investigated and the MDL study repeated.

10.1.6 The mean concentration (X), standard deviation (s) and MDL of the replicate analyses are calculated as follows for each analyte in the method:

$$X = S (x_i) / n$$

$$s = [S (x_i - X)^2 / (n-1)] ^{½}$$

$$MDL = t_{(n-1),1-a = 0.99)} * s$$

where, x_i = individual replicate measurement
n = number of replicates
t = student's t value
S = sum over n measurements

Attachment 14 provides an example MDL and contains the values for the student's t at 99% confidence. Refer to the CD for an example Excel spreadsheet to calculate MDLs.

10.1.7 A calculated MDL will be considered valid if the true concentration of the replicate spike lies within the range of 1-5 times the calculated MDL.

For example:

The calculated MDL for an analyte is 6 ug/L (standard deviation of the replicate measurement was 1.91 ug/L for 7 replicates) and the true concentration in the spiked replicates was 15 ug/L. The replicate spike is 2.5 times the calculated MDL; therefore, the MDL is acceptable.

The calculated MDL for an analyte is 6 ug/L (standard deviation of the replicate measurement was 1.91 ug/L for 7 replicates) and the true concentration in the spiked replicates was 50 ug/L. The replicate spike is 8.3 times the calculated MDL; therefore, the MDL is not acceptable. In this case the MDL study should be repeated using spiked replicates at a lower concentration, such as 25 ug/L.

10.2 MDLs Versus Practical Quantitation Levels (PQLs)

As described above, MDLs are statistically derived minimum concentrations. They are representative of a given time and set of conditions (i.e. the conditions on the day they are measured). Because these conditions – analyst variation, instrument performance, humidity, temperature, etc. may vary from day to day, a laboratory/facility should institute

a “reporting limit”. These reporting limits should be 2 to 10 times the MDL and will account for day to day variation. A reporting limit may also be called a PQL.

10.3 Accuracy and Precision Measurements

The laboratory demonstrates its capability of performing an analytical method through method validation. Method validity is established by meeting specified criteria for precision and accuracy.

The results of quality control samples created in the laboratory represent estimates of accuracy and precision for the preparation and analysis steps of sample handling.

Accuracy is the closeness of agreement between an observed value and an accepted reference value.

Precision is the closeness of agreement among a set of replicate measurements without assumption of knowledge of the true value.

This section describes the quality control information provided by each of these analytical measurements. Information on the procedures to follow in preparation of the samples or spiking solutions is described for each method and matrix in the respective method Standard Operating Procedure.

10.3.1 Method Blank

A method blank is a volume of analyte-free matrix (e.g. deionized and/or distilled laboratory water for water analyses) carried through the entire analytical procedure. The volume or weight of the blank must be approximately equal to the sample volume or weight processed. A method blank is performed with each batch of samples or one with every 20 field samples whichever is more frequent. Analysis of the blank verifies that method interferences caused by contaminants in solvents, reagents, glassware, and other sample processing hardware are known and minimized. Optimally, a method blank should be less than the PQL for all parameters unless otherwise specified in the method or SOP.

10.3.2 Accuracy Measurements

Laboratory Control Samples (LCSs) or **Laboratory Fortified Blank** (LFB) consist of aliquots of analyte-free matrices (water, sand, etc.) spiked with analytes of interest. Laboratory pure water is used to prepare most LCSs for methods for analysis of aqueous samples. LCSs provide an estimate of accuracy based on recovery of the compounds from a clean, control matrix. They provide evidence that the laboratory is performing the method within accepted guidelines generally in the absence of matrix interferences. They are prepared at a rate of one per batch of twenty or fewer samples.

Matrix Spikes/Matrix Spike Duplicates are similar to Laboratory Control Samples except the analytes used for spiking are added to a second and third separate aliquot from the field samples in a batch of analyses. They incorporate

sample matrix effects and field conditions. Matrix spikes are routinely prepared at a frequency of one MS per twenty samples for inorganic analyses when adequate sample volume is provided.

Accuracy is expressed as Percent Recovery (%R). For LCSs, percent recovery (**%**R) is calculated using the following equation:

$$\%R = (SR / SA) * 100$$

where: **SR** is the concentration determined
SA is the concentration spiked

Example: You prepare a GGA check sample for BOD analysis at 198 mg/L. Upon analysis, you obtain a calculated result of 184 mg/L. Your accuracy is expressed as:

$$\%R = (SR / SA) * 100$$

$$\%R = (184/ 198) * 100 = 93\%$$

SR = 184 mg/L
SA = 198 mg/L

For matrix spike samples, the percent recovery is calculated using the following equation:

$$\%R = (SSR-SR)/SA \times 100$$

where: **SSR** is the spiked sample determined result
SR is the original sample determined result
SA is the amount of spike added (expected)

Example: You prepare a matrix spike sample for nitrate analysis by adding 1.0 mg/L to an aliquot of your sample. Upon analysis, you obtain a result of 0.32 mg/L for your unspiked sample and 1.26 mg/L for your spiked sample. Your accuracy is expressed as:

$$\%R = (SSR-SR)/SA \times 100$$

$$\%R = (1.26-0.32)/1.0 \times 100 = 94\%$$

SSR = 1.26 mg/L
SR = 0.32 mg/L
SA = 1.0 mg/L

10.3.3 Precision Measurements

A **Replicate** is a sample that has been homogenized and split into two equal portions before the method specified sample preparation process. It measures sample precision associated with the preparation through analysis and is prepared and analyzed at a rate of one per batch or one per twenty samples (if a batch is less than twenty samples) in the inorganic laboratories.

The comparison of the values determined for a sample and its replicate (S/REP or MS/MSD) is expressed as relative percent difference (RPD). RPD is calculated using the following equation:

$$\mathbf{RPD = \frac{[S-D]}{(S+D)/2} \times 100}$$

where: S is the determined result of the original sample
D is the determined result of the replicate sample

The vertical bars in the above equation indicate the absolute value of the difference, hence RPD is always expressed as a positive value.

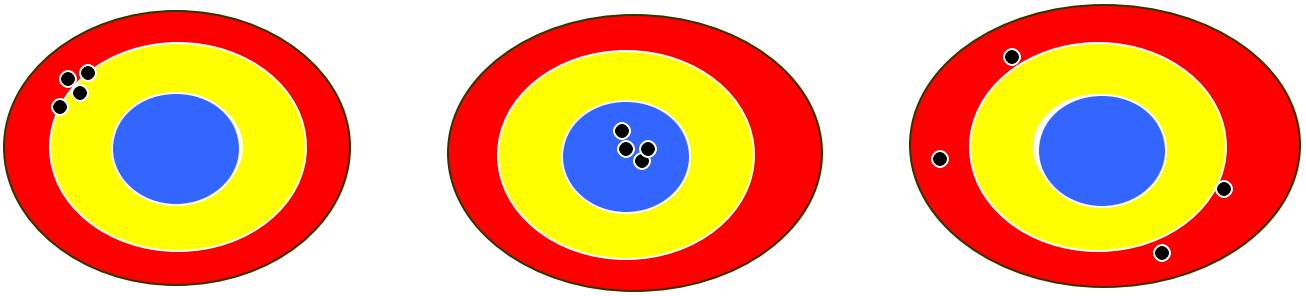
Example: You analyze a sample for nitrate and get 0.44 mg/L. You analyze a replicate of the sample and get 0.46 mg/L. Your precision is expressed as:

$$\mathbf{RPD = \frac{[S-D]}{(S+D)/2} \times 100}$$

$$\mathbf{RPD = \frac{[0.44-0.46]}{(0.44+0.46)/2} \times 100 = 4.4\%}$$

S = 0.44 mg/L
D = 0.46 mg/L

Another way to look at accuracy and precision is to think of them in terms of a bull's eye chart.



Good Precision Poor Accuracy	Good Precision Good Accuracy	Poor Precision Poor Accuracy

10.4 Statistical Control Limits

Statistically derived laboratory limits serve as a tool for evaluating method performance, for evaluating individual analyst performance and for monitoring the effects of changes to the analytical methods. Statistically derived QC limits should be calculated as 3 standard deviations from the mean recovery of a minimum of twenty data points. This may be done on an annual basis for Laboratory Control Samples and/or matrix spike/matrix spike duplicates if sufficient data is available. A minimum of twenty data points are required for a given analytical procedure and sample matrix prior to generating statistical control limits. Until twenty data points are available, recommended EPA recovery limits must be used if available. Data points shall be chosen at random. All data points used in the determination must be taken from data where all routine applicable QC criteria have been met for the analysis.

The percent recovery is calculated for each spiked analyte. The average percent recovery (X) and the standard deviation (s) are calculated for the group of samples.

Both upper and lower warning limits and upper and lower control limits are established to interpret performance. Warning limits express a narrower confidence interval and are used to warn the technician of possible system inconsistencies or failures, before an out-of-control event occurs. Control limits express the outer limits of accepted method variability. Control limits and warning limits are reviewed periodically against performance. Based on statistical considerations, an evaluation is made to determine whether the control limits need to be revised.

<u>Warning Limits</u>

When not mandated by the method, warning limits should be the mean ±2 standard deviations or a 95% confidence interval, where:

The mean percent recovery and standard deviation are calculated as follows:

Mean(P) = $$\frac{1}{n}\sum_{i=1}^{n} X_i$$

Standard Deviation(s) =

$$S^2 = \frac{\sum_{i=1}^{n} X_i^2 - \left(1/n\right)\left(\sum_{i=1}^{n} X_i\right)^2}{n-1}$$

where: X = individual values
N = total number of values

Recovery warning limits are to be calculated using the following formulas:

$$UWL = P + 2s$$
$$LWL = P - 2s$$

where: UWL = Upper Warning Limit
LWL = Lower Warning Limit

Control Limits

Unless otherwise specified by the analytical method in use the 99% confidence interval is used as the control limits, which is defined as the mean ±3 standard deviations. Where the method specific ranges have been determined, control limits should be similar to the method limits. Control limits are established as follows using the mean and standard deviation as above:

$$UCL = P + 3s$$
$$LCL = P - 3s$$

where:
UCL = Upper Control Limit
LCL = Lower Control Limit
P = Mean Percent Recovery
s = Standard Deviation

The control limits and warning limits used to evaluate a sample should be those in place at the time that the sample was analyzed. Once limits are updated, the limits should apply to all subsequent analyses.

10.5 Control Charts

Control charts are quality control tools that graphically display the QC parameters over time. The lab may generate control charts as a means to identify method or analyst performance issues.

10.5.1 Accuracy

Accuracy charts can be used for Laboratory Control Sample recovery. The percent recovery is plotted onto the graph where:

the x-axis is the sample ID; and
the y-axis is the range of percent recoveries.

10.5.2 Precision

Precision charts can be used for LCS/LCSD and MS/MSD comparison. The relative percent difference is plotted on the graph where:

the median, zero, represents 0% difference
the x-axis is the number of data points per chart; and
the y-axis is the range of relative percent differences.

Control chart limits may be evaluated using the following guidelines.

Suspicious/Out-of-Control Events

Plotting and connecting successive data points on control charts enables the laboratory to detect many types of suspicious and out-of-control situations. These events can be caught by monitoring the following: outliers (suspicious and out-of-control), runs (suspicious), trends (suspicious), and periodicity (suspicious).

Outliers

There are two types of outliers: any particular point that falls outside the control limits or any point that falls outside the warning limits. A point that falls outside the control limits is classified as an out-of-control event; a point that falls outside the warning limits is classified as a suspicious event.

Runs

A run is defined as a series of points that line up on one side of the central line (the mean). Any run that has a length of seven points is indicative of a potential abnormality in the process, a suspicious event. A run can suggest several potential problems such as a leak in the system, elevated contamination, or incorrect dilutions of standards.

Trends

A trend is defined as a series of points that are marked by an unbroken rise or fall. Any trend with a length of five points is classified as a suspicious event. A trend may indicate a change in instrument sensitivity due to a dirty source or injection port or standard degradation, to name a few.

Periodicity

Periodicity is a term used to describe a recurring pattern of change over equal intervals. This occurrence may be of any length or amplitude; thus, careful observation of the control chart is necessary.

Refer to Attachment 15 for an example control chart.

11.0 ANALYTICAL METHODS & SPECIFIC QC

Analytical procedures are detailed descriptions of any and all processing, preparation and analysis of samples in the laboratory. In some instances, data format, presentation and delivery are also described. All analytical procedures shall be conducted in strict adherence to the QA Manual and written Standard Operating Procedures that have been reviewed and approved by the appropriate personnel.

The method SOPs should contain specific requirements for all QC samples. Table 11-1 lists minimum DEP requirements for QC parameters. Other analytical issues are described below:

11.1 Settleable Solids (SS)

- Do not estimate floating material as settleable matter.
- Record the results in mL/L/hr.

11.2 Total Residual Chlorine (TRC)

- An amber bottle should be used because chlorine dissipates rapidly in sunlight.
- The bottle should be completely filled because chlorine dissipates with the atmosphere.

11.3 Biochemical Oxygen Demand (BOD)

- Sample and dilution water must be 20 °C.
- Initial DO must be < 9 mg/L.
- Blank depletion must be <0.2 mg/L.
- Sample residual DO must be > 1 mg/L.
- Sample DO depletion must be >2 mg/L.

11.4 Total Suspended Solids (TSS)

- Excessive solids on the filter may leave a residue that can form a water trapping crust. To guard against this problem, the selected sample volume should produce no more than 200 mg of residue.

11.5 pH

- The pH meter should be capable of reading to at least 2 digits beyond the decimal place, i.e. 7.43, not 7.4.
- The pH probe should have temperature compensation.
- The pH meter should be warmed before use for at least 30 minutes.
- The pH meter shall be standardized with at least two buffers that will bracket the readings of the samples.
- A third buffer should be read as a check sample.
- The probe should be rinsed with DI water between samples and buffers.

11.6 E. coli / Fecal coliform

- All equipment must be sterilized with autoclave, UV light, alcohol or flame.

11.7 Temperature

- Temperature must be measured on-site.
- Temperature conversions:

$$°C = \frac{(°F - 32) \times 5}{9} \qquad °F = \frac{(°C \times 9)}{5} + 32$$

11.8 Phosphorus

- Samples must be digested prior to analysis

Table 11-1

Parameter	Calibration/ Standardization	QC Standards	Duplicates/ Replicates	Spikes	Blanks
Ammonia	As per method	1/10 Tests	1/10 Tests	1/Yr	1/each
BOD	Meter before each use	1/10 Test with GGA	1/10 Tests (*)	N/A	2 or 3/each
Chlorine Residual Meter	As per method	1/year (DMR_QA sample is acceptable.)	1/10 Tests	N/A	1/each
COD	As per method	1/10 Test	1/10 Tests	1/Yr	1/each
E. Coli	See additional QC requirements in the method.	POS control/each test	1/10 Tests	N/A	1/each
Fecal Coliform	See additional QC requirements in the method.	POS control/each test	1/10 Tests	N/A	1/each
Nitrate Nitrogen	As per method	1/10 Tests	1/10 Tests	1/Yr	1/each
pH	Minimum 2 point calibration each use	(3^{rd} buffer) 1/each	1/10 Tests	N/A	N/A
Phosphorus	As per method	1/10 Tests	1/10 Tests	1/Yr	1/each
Settleable Solids	N/A	N/A	N/A	N/A	N/A
Total Suspended Solids	Constant weights after filtering sample	1/year (DMR_QA sample is acceptable.)	1/10 tests	N/A	N/A

(*) Different dilutions of the same field sample are not counted as part of the 10 tests. Only count separate field samples.

12.0 DOCUMENTATION & RECORDS MANAGEMENT

Records are the means by which an organization documents its operations and activities. They are an integral part of the Quality Assurance program since they provide documented evidence for program functionality and necessary information for proficiency testing and quality assurance audits. All information related to the quality assurance practices outlined in this manual shall be contained in records. This shall include, but not be limited to:

- standard operating procedures
- results of instrument calibrations
- analysis of quality control samples
- analysis of samples
- sample custody and disposal
- preparation of standards
- COC documentation
- analytical records
- corrective action reports
- audits and inspections

12.1 General Recordkeeping

12.1.1 All documentation must be accurate, legible, complete and recorded in a timely manner using indelible ink. No measurements are to be filled in ahead of time even if the measurement is always the same. During the actual analysis it may be determined that a different quantity is needed.

12.1.2 All data and/or results that are recorded and/or entered must be a true and accurate representation of the measured values and must be validly quantitated.

12.1.3 If an error is made, a single line is used to cross out the incorrect entry. The original entry must remain readable. The correction must be initialed and dated and given an explanation for the change. The use of white out is prohibited on all raw data, including instrumental hardcopy.

12.1.4 When blank space is left after all information has been recorded on a logbook page or in other documentation, that blank space should be "Z'd" out. Use a single line through the space; initial and date the cross out.

12.1.5 All blocks should be filled in on pre-printed forms. Header information must be complete. All columns and units of measure must be identified.

12.2 Standard Operating Procedures

Standard Operating Procedures (SOPs) are written for specific procedures or operations. Complex tasks of inspection, testing, calibration, monitoring, maintenance, data handling, and quality control as well as methods utilized in the laboratory are specified and documented by SOP. More detailed information regarding SOPs can be found in Section 4.0. All personnel are required to follow SOPs when a specific operation or method is being utilized.

12.3 Sample Tracking

Samples are tracked from the time they are received, through storage, preparation, analysis, and final disposition. Proper sample identification must be established during sample collection. This information must be clearly and permanently written on a label and attached to the sample. In addition, a Chain-of-Custody must be initiated with the appropriate information recorded if the sample analysis is conducted by another individual. Samples must also be properly preserved and stored.

12.4 Standards

Standards preparation is documented in the standards logbooks maintained by the laboratory. All information needed to maintain proper traceability of standards is recorded in the appropriate standards logbook by the individual preparing the standard. More complete information regarding standards is provided in Section 6.0.

12.5 Maintenance Logbooks

A maintenance logbook should be kept for all instruments. Each instrument should have a unique page in the maintenance logbook. In the logbook, an analyst records initial instrument setup, routine preventive maintenance, outside contractor services, instrumental malfunctions and repair performed, dates taken in and out of service, and resolutions. Instrument logs not only describe the instrument's history, but can be helpful when troubleshooting. Additionally, runlogs may describe problems noted, maintenance performed and return to control. *Refer to Attachment 16 for an example maintenance logbook.*

12.6 Bench Logbooks (preparation & analysis)

All data pertinent for sample preparation and analysis should be recorded by the laboratory staff in bound notebooks. *Refer to Attachment 17 for example benchsheets*. It should contain the following information:

- Sample identification numbers
- Date of preparation and analysis
- Method reference
- Analyst's initials
- Preparation weights and/or volumes (initial and final)
- Reagent/solvents used including manufacturer and lot number
- Relevant blank
- Spike data including the serial reference number
- Notable observations
- Entire sequence of samples, including the calibration curve
- Identification of the instrument
- Acceptability of the results in the context of the QC system.
- Amount analyzed and any dilution of the original sample and/or extract
- Any data relevant to the calculations

13.0 DATA REDUCTION, VALIDATION AND REPORTING

All analytical data generated within the laboratory should undergo a well-defined, well-documented multi-tier review process.

13.1 Data Reduction/Documentation

All raw data are recorded on a standardized recording form. *Refer to Attachment 17 for example benchsheets.* The analyst conducting the analysis records, at a minimum:

- method used
- date of analysis
- raw data readings
- calculations
- final results
- analyst's initials or signature

Any deviations from standard data reduction procedures must also be recorded.

All data and/or results that are recorded and/or entered must be a true and accurate representation of the measured values and must be validly quantitated.

All documentation must be accurate, legible, complete and recorded in a timely manner using indelible ink. No measurements are to be filled in ahead of time even if the measurement is always the same. During the actual analysis it may be determined that a different quantity is needed.

If an error is made, a single line is used to cross out the incorrect entry. The original entry must remain legible. The correction must be initialed and dated and given an explanation for the change. The use of white out is prohibited on all raw data, including instrumental hardcopy.

When blank space is left after all information has been recorded on a logbook page or in other documentation, that blank space should be "Z'd" out. Use a single line through the space; initial and date the cross out.

All blocks should be filled in on pre-printed forms. Header information must be complete. All columns and units of measure must be identified.

For data, which are reduced by manual calculations, an example calculation must be documented in a laboratory notebook or on an analyst's worksheet.

All reduced data must be evaluated by the analyst using the QA acceptance criteria found within each analytical method SOP.

13.2 Significant Figures

To avoid reporting results that are inaccurate or deceiving, "significant figures" are used. Significant figures give an indication of the reliability of the analytical method used. Reported values should only contain those values that are significant. A value is made up of significant figures when it contains all digits known to be true and one last digit in doubt. For example, in the number 21.2, the 21 is a firm value, but the 2 may be a one or a three. This value is in doubt. This number contains 3 significant figures. Refer to the following table for required significant figures.

Parameter	**Precision for DMRs**	**Example**
BOD or CBOD	No digit after decimal point, except if the value reported is <10, then, one digit after the decimal place	28 mg/L or 8.5 mg/L
Chlorine Residual	Two digits after decimal point	0.51 mg/L
Coliform (Fecal or *E. coli*)	No digits after decimal point	50/100 ml
TKN, NH_3, NO_3, NO_2	One digit after decimal point	17.6 mg/L
DO	Two digits after decimal point	7.35 mg/L
Settleable Solids	One digit after decimal point	5.1 ml/L
Metals	One digit after decimal point	436.3 ppb
pH	Two digits after decimal point	7.00 pH units
Suspended Solids	No digit after decimal point Except if the value reported is <10, one digit after the decimal place	22 mg/L or 7.8 mg/L
Temperature	One digit after decimal point	17.2°C

Standard Methods specifies that for calculations involving multiplication or division, the result of the calculation should be have the same number of significant digits as the factor that has the least number of significant digits. If the result expressed in common notation (e.g. 2,348) has more significant digits than the factor with the fewest significant digits, the result should be displayed in scientific notation (e.g. 2.3×10^3). While this is technically correct, the PCS (DEP) system will not accept numbers in scientific notation. Therefore, for purposes of DMR data entry, the results of calculations involving multiplication or division that should be, by the convention in *Standard Methods*, displayed in scientific notation, will be displayed with the correct number of non-zero significant digits and with the remaining places needed to display the number in normal notation filled with zeros.

For example, (this is a possible "real world" example) A POTW has an effluent BOD concentration of 22 mg/L and treats 2.582 MGD on a given day. The pounds of BOD discharged are calculated using the formula:

Quantity (in pounds) = Concentration (in mg/L) * Flow (in MGD) * 8.34

Plugging in the numbers:

Quantity (in pounds) = 22 mg/L * 2.582 MGD * 8.34 = 473.74536 pounds

Using the *Standard Methods* convention, that the answer should have the same number of significant digits as the factor with the fewest significant digits (22 has 2 significant digits), the answer should be displayed as 4.7×10^2 pounds. The convention we will adopt will display the answer as 470 pounds. Although this "breaks" the convention because the zero is considered a "significant" digit, this is more understandable than the "correct" alternative. Most operators would round the number off to 474 pounds, but that, according to the convention would be even more incorrect.

13.3 Rounding

The following rounding rules shall be used when determining the correct number of significant figures. The number of significant figures varies with the test performed (see table above).

All digits are used in the calculation, then, the final number is rounded, using the following guidelines. Numbers that are not significant must be dropped by rounding off. If the digit 6, 7, 8, 9 is dropped round up one unit. If the digit 0, 1, 2, 3, 4 is dropped, do not change the preceding digit. If the digit 5 is dropped, round to the nearest even number.

For example:

3.57 is rounded to 3.6
2.41 is rounded to 2.4
4.44 is rounded to 4.4
7.35 is rounded to 7.4 (the nearest even number)
7.65 is rounded to 7.6 (the nearest even number)

13.4 Data Validation

The analyst who completes the analysis assembles:

- all relevant raw data and results
- strip chart recordings
- spreadsheet calculations
- instrument settings and/or other essential information to data interpretation

The calculations and final results recorded on the recording forms or laboratory notebook are reviewed and initialed by a second qualified reviewer. The reviewer checks the data recording forms and completed worksheet for accuracy, consistency, and fulfillment of quality control criteria. All manual calculations are checked and 10% of all spreadsheet calculations are checked. The remainder of spreadsheet calculations are spot checked for potential anomalies. The reviewer approves the worksheet by initialing it.

14.0 PREVENTIVE MAINTENANCE

To minimize downtime and interruption of analytical work, preventive maintenance is routinely performed on each analytical instrument. Designated laboratory personnel are trained in routine maintenance procedures for all major instrumentation. When repairs are necessary, they are performed by either trained staff or instrument manufacturer service personnel.

SOPs are written for each instrument that cover basic operation and maintenance procedures. Detailed logbooks documenting preventive maintenance, non-routine maintenance and repairs are also maintained for each instrument. The following are brief summaries of maintenance for each major instrument.

14.1 Preventive Maintenance - General Laboratory Areas

- Clean and calibrate balances biannually (minimum) by an outside source
- Check balance calibration each day of use
- Clean balance pan prior to each use
- Calibrate automatic pipettes with each use
- Calibrate thermometers yearly against an NIST traceable thermometer
- Record refrigerator, freezer, and oven temperatures each weekday
- Clean, check, calibrate to manufacturers' specifications all pH, DO, conductivity, spectrophotometers, and turbidity meters annually (recommended) using an outside service
- General housekeeping: keep counter tops, hoods, and floors clean
- Check airflow in hoods once a week

14.2 DO Probe Preventive Maintenance

- Visually inspect the probe membrane for tears, oily residues, or fingerprints
- Inspect for air bubbles under the membrane. If any of the above are present, replace the membrane cap
- Replace electrolyte solution when membrane cap is replaced

15.0 PERFORMANCE AND SYSTEM AUDITS

Proficiency Test samples (PTs) may be analyzed periodically to verify method accuracy. These PT samples may be external (e.g. DMR-QA) or internal (prepared or purchased). DMR-QA samples must be analyzed annually.

15.1 Proficiency Test (PT) Samples – DMR-QA - Annual

EPA requires some laboratories to perform annual Quality Assurance tests on samples containing unknown quantities of specific analytes. The laboratory must obtain the samples from an approved PT provider. The samples are analyzed for prescribed analytes. The quantities of analytes found in the samples are reported to the contractor who issues a report to the analyzing laboratory and to the Maine DEP stating whether the analyses performed gave results within allowable limits. If the result from any analysis falls outside the acceptable limits, the analyzing laboratory should determine the reason for failing the test and implement corrective actions. This may include ordering another sample for reanalysis to determine if the problem has been found and solved.

15.2 Periodic Internal Audits

In the event that the overseeing regulatory agency requires an internal audit, the following would apply: internal auditing is conducted by a designated person. These audits should focus on performance relative to an SOP. Internal audits take two forms - performance audits and systems audits.

15.2.1 Performance Audits involve analysis of blind spikes obtained from an outside service. These samples may be performed if results for a particular method are in question. Analyzing a known sample is a good way to determine whether a problem may or may not exist.

15.2.2 Systems Audits consist of a thorough review of procedures and documentation to confirm that work is being performed in accordance with this Manual and SOPs. They should be performed by an individual with knowledge of laboratory procedures. Audit checklists may be used to ensure that all points are addressed and documented.

Audit checklists may cover at least the following areas:

- Personnel qualifications and training records
- Adequacy of laboratory facilities, including work space, lighting, ventilation, and supplies
- Maintenance and calibration recordkeeping for analytical equipment
- General operations, including glassware cleaning, inventory and checking of reagents and standards, and storage procedures
- Recordkeeping, including sample log-in and tracking, traceability of standards, control charts, and raw data recording and tracking.

A summary of the audit findings should be written by the auditor. Any corrective actions to be taken should also be explained. The report should be filed for future reference.

16.0 CORRECTIVE ACTION

For most laboratory situations, problem identification, corrective action, and resumption of operation and/or return to control occurs at the bench, with documentation written directly in the appropriate logbooks or benchsheets. These occurrences include events where laboratory quality control criteria have been exceeded but which can be corrected without compromising the analytical results or delaying the preparation or analytical process.

For other situations, problem identification, corrective action, and resolution are tracked via Corrective Action Reports (CARs). CARs (*Refer to Attachment 18 for an example CAR*) should be initiated:

- **When Quality Control criteria are not met** - These QC criteria include, but are not limited to, blanks, LCSs, spikes, ICVs/CCVs. *Note: it may not be necessary to initiate a CAR in each case, especially if data is rejected, but the corrective action should be documented on the raw data, logbook or other analyst records.

- **When laboratory SOPs are not followed** - This includes all aspects of laboratory operations from receipt to reporting.

- **When you are concerned that a major problem or potential problem exists in the laboratory.**

The underlying purpose of the corrective action process is to identify instances that may adversely affect the data. Corrective actions also help:

- **To help standardize the laboratory's procedure for handling events that require corrective action** - Every situation should be evaluated individually, but there are some basic guidelines that should be followed.

- **To record actions taken when SOPs are not followed so that the data produced is supported with a documented sequence of events.**

- **To document occurrences in the lab that** may **affect the integrity of laboratory records**

- **To provide a learning tool for individuals involved in the problem investigation and corrective action plan**

- **To provide a means for tracking recurring problems that** may **need further investigation into the root cause of the problem.**

16.1 Problem Identification

The analyst generating the data is responsible for reviewing all results against the established limits. Any deviations are immediately evaluated as potential out-of-control events. Specific examples of some out-of-control events may be: Laboratory Control Sample (LCS) failures, blank contamination, poor precision, prep errors, missed holding

times, calibration failures, and matrix spike failures. If data **are** outside accepted limits, the analyst should review and evaluate the data and all associated Quality Control elements together before making a decision as to the acceptability of the data. Each individual method SOP contains corrective action tables to help guide analysts in making these decisions. Once all QC items have been considered, the analyst should immediately take the appropriate actions.

16.2 Corrective Action

The appropriate action may differ with each situation. In some cases data may be reported, but may be reanalyzed in other cases. Making new reagents and standards may be necessary if the standardization is suspect. The corrective actions listed in every method SOP may rely on analyst experience to make sound scientific judgements. These decisions may be based upon whether there is remaining analyte holding time.

The return of control of the measurement, confirmed by data within acceptable limits as set by the manufacturer, SOP or policy, prove completion of corrective action.

The following are some examples of possible corrective actions that may be taken for several out-of-control events. In all cases the corrective action taken shall be documented in a logbook or in a CAR, whichever is appropriate.

16.2.1 Method Blanks (when required by the SOP)

Corrective action is taken whenever the analyte is detected in the method blank above the reporting limit or PQL (Practical Quantitation Limit):

- Check all calculations
- If samples are non-detect, the high blank has not biased the sample. Report data with no qualifier
- If sample concentrations are significantly greater than the blank level (i.e. 10X), the sample is not significantly impacted by the blank. Report sample results with a qualifier indicating the blank level
- If sample concentrations are between the PQL or reporting limit and 10X the blank level, they should be reanalyzed and/or reextracted/redigested
- Investigate the source of the problem

Example: During a nitrate analysis, a method blank and ten samples are analyzed. The reporting limit for nitrate is <0.05 mg/L. The following results are obtained:

Blank – 0.072 mg/L	Sample 1 - <0.05 mg/L	Sample 2 – 0.34 mg/L
Sample 3 – 0.89 mg/L	Sample 4 – 3.7 mg/L	Sample 5 – 0.13 mg/L
Sample 6 - <0.05 mg/L	Sample 7 – 0.064 mg/L	Sample 8 – 10.2 mg/L

The blank result is greater than the reporting limit, indicating possible contamination for the process. Using the above criteria, Samples 1and 6 would not have to be reanalyzed because they are non-detect, thus showing no high bias from the blank. Samples 3, 4, and 8 are greater than ten times the blank level (0.072 x 10 = 0.72) and, therefore, are not significantly impacted by the blank contamination. These do not have to be reanalyzed. That leaves samples 2, 5 and 7 that are in the same range as the blank. These should be reanalyzed.

16.2.2 Laboratory Control Samples (when required by the SOP)

The % recovery of Laboratory Control Samples (LCS) is calculated. Corrective action is taken whenever the % recovery is outside the established acceptance criteria. The following corrective actions are taken when required:

- Check calculations to assure there are no errors
- Check internal standard and spiking standard solutions for degradation, contamination, etc., and check instrument performance
- If LCS fails high and samples are non-detect, report associated data with a narrative explanation
- Reanalyze other samples associated with a failed LCS, if available

16.2.3 Matrix Spike and Matrix Spike Duplicates (when required by the SOP)

The % recovery of Matrix Spike and Matrix Spike Duplicates are calculated. The following corrective actions are taken when required:

- If all QC associated with a sample is within acceptance limits (method blank and LCS spike recoveries), the problem may be attributed to a matrix effect

16.2.4 Calibration

Individual methods specify calibration frequency and criteria. If the calibration curve is suspect, the following steps should be taken:

- Check instrument for contamination
- Check instrument for incorrect operating conditions
- If no source of the problem is identified, then a complete initial calibration must be performed

16.3 Documenting Corrective Action

All corrective actions should be documented on a Corrective Action Report as soon as possible after problem identification. The following information must be documented on the CAR:

- When and where the out-of-control event occurred
- The person who discovered the out-of-control event
- What analysis was being performed
- What samples are affected by the out-of-control event
- A brief description of the out-of-control event
- Steps taken to investigate the out-of-control event
- The probable cause of the out-of-control event
- Any corrective actions taken, both immediate and long term to prevent reoccurrence

16.4 Review of Corrective Action

All corrective action reports must be reviewed by the appropriate management personnel. Historical corrective action reports may be reviewed to identify long-term trends or recurring problems. The root cause of the problem shall be identified as: lack of organization, lack of resources, lack of training, lack of time, lack of discipline or lack of top management support. All documentation associated with the CAR, i.e. raw data or reissued reports shall be filed.

Attachment 1
Terms, Definitions & Acronyms

Accuracy:	The closeness of agreement between an observed value and an accepted reference value. When applied to a set of observed values, accuracy will be a combination of a random component and of a common systematic error (or bias) component.
Aliquot:	A measured portion of a sample taken for analysis.
Analyte:	The specific component or constituent that the analytical measurement seeks to determine.
Batch:	A group of samples which are treated similarly with respect to the sampling or the testing procedures being employed and which are processed as a unit. For QC purposes, if the number of samples in a group [first] is greater than 20, then each group [successive] of 20 samples or less will all be handled as a separate batch.
Blank:	See Equipment Rinsate, Method Blank, Trip Blank, Field Blank, Calibration Blank.
Blind Sample:	A sample submitted for analysis whose composition is known to the submitter but unknown to the analyst.
Calibration:	The process of establishing the relationship between instrumental response and known traceable quantities of analytes of interest.
Calibration Blank:	A quality control sample prepared in the same manner as calibration standards with the exception of the addition of the analytes of interest. A calibration blank is used to establish solvent/reagent and system contributions to the analytical result.
Calibration Verification	The process of analyzing a mid-level calibration standard to verify the validity of the calibration curve.
Comparability:	Comparability is a qualitative parameter expressing the confidence with which one data set can be compared to another. Comparable data are produced through the use of standardized procedures and techniques.
Completeness:	Measure of the amount of valid data obtained from a measurement system compared to the amount that was expected to be obtained under correct normal conditions
Composite Sample:	A collection of individual samples obtained at set intervals over a period of time.
Continuing Calibration:	The process of analyzing standards periodically to verify the maintenance of calibration of the analytical system.

Control Chart:	A graphical plot of test results with respect to time or sequence of measurement, together with limits within which they are expected to lie when the system is in a state of statistical control.
Control Limit:	A range within which specified measurement results must fall to signify statistical control. A process is considered in control if data falls within the prescribed limits. A process is considered "out-of-control" if data falls outside the established control limits. These data are considered suspect and require corrective action including, but not limited to, qualification of the data.
Data Quality: Objective	Qualitative and quantitative statements that define the appropriate type of data, and specify tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions.
Data Validation:	The internal process of review by which data are shown to be valid as evidenced by the soundness of the analytical system.
Deionized Water:	Water that has been deionized to produce reagent grade water. Refer to reagent grade water.
Dilution:	Lowering the concentration of a solution by adding more solvent (usually distilled water).
Dry Weight:	The weight of a sample based on percent solids. The weight after drying in an oven following lab protocol.
Effluent:	The output or discharge from a water treatment process.
Equipment Blank:	A field blank used to verify the effectiveness of equipment decontamination procedures. Laboratory deionized water is passed over sampling equipment after decontamination, collected, and analyzed by the lab.
Field Blank:	Samples of analyte-free media (generally water) taken from the laboratory to the field as: 1) distinct aliquots in the same containers used to collect samples with the appropriate preservative reagents added, or; 2) a single reserve to be aliquoted in the field into the appropriate containers with the appropriate preservatives for the parameters of interest. The intent of the field blank is to ascertain and document any contamination attributable to shipping, field handling procedures and potentially to ambient conditions.
Field Duplicate:	Independent samples that are collected as close as possible to the same point in space and time. They are two separate samples taken from the same source, stored in separate containers and analyzed independently. These duplicates are useful in documenting the precision of the sampling process.
Field Sample:	A portion of material received by the laboratory to be analyzed, that is contained in single or multiple containers and identified by a unique field ID number.

Grab Sample: A single sample of wastewater.

Holding Time: The elapsed time expressed in days (except for parameters requiring analysis in ≤ 48 hours) from the date of sample collection by the field personnel until the date of its processing/analysis. Holding time requirements are dictated by the EPA Federal Register 40CFR Part 136, Table II.

Homogeneity: The degree to which a property or substance is evenly distributed throughout a material.

Influent: Wastewater or other liquid flowing into a reservoir, basin, treatment process or treatment plant.

Instrument Detection Limit: Smallest signal above background noise that an instrument can detect at a 99% confidence level that the analyte concentration is greater than zero. The IDL does not consider any effects that the sample matrix, handling or preparation may have.

Initial Calibration: The process of analyzing standards, prepared at specified concentrations, to define the quantitative response, linearity and dynamic range of the instrument to the analytes of interest. Initial calibration is performed whenever the results of a continuing calibration do not conform to the requirements of the method in use or at a frequency specified in the method.

Lab Control Sample: A control sample whose matrix is of known composition or analyte-free matrix spiked with a known concentration of analytes of interest. Laboratory control samples are handled using the same preparation, reagents, and analytical methods employed for field samples. Laboratory Control Samples are utilized as indicators of the accuracy of the analysis.

Lot: A quantity of bulk material of similar composition processed or manufactured at the same time.

Matrix: The component or substrate (e.g. surface water, drinking water) which contains the analyte of interest.

Matrix Duplicate: An intralaboratory split sample which is used to document the precision of a method in a given sample matrix.

Matrix Spike: Aliquot of sample fortified (spiked) with known quantities of specified analytes and processed through the entire procedure in order to indicate the appropriateness of the method for the matrix by measuring recovery.

Matrix Spike Duplicate: Intralaboratory split samples spiked with identical concentrations of target analyte (s). The spiking occurs prior to sample preparation and analysis. They are used to document the precision and bias of a method in a given sample matrix.

Meniscus: The curved top of a column of liquid in a small tube. When the liquid wets the sides of the container (as with water), the curve forms a valley.

Method Blank: An analyte-free matrix to which all reagents are added in the same volumes or proportions as used in sample processing. The method blank should be carried through the complete sample preparation and analytical procedure. The method blank is used to document analyte contribution resulting from the analytical process. Acceptable levels of contamination are defined in individual SOPs and/or by project specific data quality objectives.

Method Detection Limit: The statistically derived minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. Method detection limits are determined using replicate spike samples prepared by the lab and taken through all preparation and analysis steps of the method. The method detection limit is calculated using the appropriate Student's t parameter times the standard deviation of a series of spiked samples.

NPDES permit: National Pollutant Discharge Elimination System permit, the legally enforceable document that sets forth the terms, conditions and limitations by which a wastewater treatment system must operate. The NPDES is authorized by both state and federal law and it allows stiff civil and criminal penalties for failure to comply. NPDES permits must be obtained for all point source discharges into US waterways. NPDES permits are administered in Maine by the DEP. The program is referred to as MEPDES.

Performance Audit: A process to evaluate the compliance of actual laboratory practices with relevant project requirements, regulations, contract specifications or internally stated standard operating procedures and practices.

Precision: The agreement among a set of replicate measurements without assumption of knowledge of the true value. Precision is estimated by means of duplicate/replicate analyses. These samples should contain concentrations of analyte above the MDL, and may involve the use of matrix spikes. The most commonly used estimates of precision are the relative standard deviation (RSD), when two or more samples are available and the relative percent difference (RPD), when only two samples are available.

Preservative: A chemical or reagent added to a sample to prevent or slow decomposition or degradation of the analyte to be tested.

Proficiency Test: A process to evaluate the proficiency of an analyst or laboratory by evaluation of the results obtained on known test materials.

Protocol: A stated plan that clearly defines the objectives, methods and procedures for accomplishing a task.

PQL: Practical Quantitation Limit; a value three to five times the Method Detection Limit.

Quality Assurance Program: A system of policies and procedures whose purpose is to ensure, confirm and document that the product rendered fulfills the requirements of Facility Name. Quality Assurance includes quality planning, quality control, quality assessment (auditing), quality reporting and corrective action.

Quality Control: A system of checks and corrective measures, integrated with the activities that directly generate the product or service, that serves to monitor and adjust the process to maintain conformance to predetermined requirements.

Reagent-Grade Water: Water that has no detectable concentration of the element or compound to be analyzed at the detection limit of the method and water that is free of substances that interfere with the method. This determination is dependent on the analytical test (detection limit and interferences) to be used. See deionized water.

Replicate: Aliquots of sample taken from the same container and analyzed independently.

Reporting Limit: The level at which method, permit, regulatory and client specific objectives are met. The reporting limit may never be lower than the statistically determined MDL, but may be higher based on any of the above considerations. Reporting limits are corrected for sample amounts, the dry weight of solids, and instrument dilution factors, unless otherwise specified.

Sensitivity: Capability of methodology or instrumentation to discriminate between samples having different concentrations or containing differing amounts of an analyte.

Significant Figures The number of digits in a value that are justified by the accuracy and precision of the method being used. A value is made up of significant figures when it contains all digits known to be true and one last digit in doubt.

Spike: Aliquot of sample or deionized water fortified (spiked) with known quantities of specified analytes and processed through the entire procedure in order to indicate the appropriateness of the method for the matrix by measuring recovery. See also matrix spike and Lab Control Sample.

Standard: A substance or material the properties of which are known with sufficient accuracy to permit its use to evaluate the same property in a sample.

Standard Operating Procedure: A written document that details the method for an operation, analysis, or action with thoroughly prescribed techniques and steps that outline expected limits of achievement and will produce consistent performance

with repetitive use. This document must be officially approved as the method for performing certain routine or repetitive tasks.

Superintendent: Senior responsible official. This may be a Chief Operator, Lab Supervisor, Town Manager, or Mill Manager.

Systems Audit: An on-site inspection or assessment of a laboratory's quality system or one of its components.

Titration: Process in which an accurate volume of a titrant (known concentration) is dispensed into a known volume of sample (unknown concentration)

Traceability: The ability to trace the source and accuracy of a material (i.e., standard) to a recognized primary reference source such as the National Institute of Standards and Technology (NIST) or USEPA. Also, the ability to independently reconstruct and review all aspects of the measurement system through available documentation.

Trip Blank: A sample of analyte-free media taken from the laboratory to the sampling site and returned to the laboratory unopened. A trip blank is used to document contamination attributable to shipping and field handling procedures. This type of blank is useful in documenting contamination of volatile organics samples.

Validation: The internal process of review by which data are shown to be valid as evidenced by the soundness of the analytical system and successful meeting of the DQOs (not to be confused with data validation by an outside independent source).

Warning Limits: The limits (typically 2 standard deviations either side of the mean) within which most analytical results are expected to lie with a 95% probability while the system remains in a state of statistical control.

ACRONYMS

AA	Atomic Absorption
ACS	American Chemical Society
CAR	Corrective Action Report
CCB	Continuing Calibration Blank
CCC	Calibration Check Compounds
CCV	Continuing Calibration Verification
CDP	Continued Demonstration of Proficiency
COC	Chain-of-Custody
DMR	Discharge Monitoring Report
GC	Gas Chromatograph
GCMS	Gas Chromatograph Mass Spectrometer
GFAA	Graphite Furnace Atomic Absorption
GLP	Good Laboratory Practices
HDPE	High Density Polyethylene
IC	Ion Chromatography
ICB	Initial Calibration Blank
ICP	Inductively Coupled Plasma (Spectrophotometer)
ICV	Initial Calibration Verification
IDL	Instrument Detection Limit
IDP	Initial Demonstration of Proficiency
LCS(D)	Laboratory Control Sample (Duplicate)
LRS	Linear Range Standard
MDL	Method Detection Limit
MS(D)	Matrix Spike (Duplicate)
NBS	National Bureau of Standards
NIST	National Institute of Standards Traceability
OSHA	Occupational Health & Safety Administration
PQL	Practical Quantitation Limit
PT	Proficiency Test
QA	Quality Assurance
QAO	Quality Assurance Officer
QAM	Quality Assurance Manual
QC	Quality Control
RPD	Relative Percent Difference
RSD	Relative Standard Deviation
SD	Sample Duplicate
SOP	Standard Operating Procedure

Attachment 2

Permit Requirements

SAMPLE LOCATION	SAMPLE TYPE	SCHEMATIC REFERENCE	PARAMETERS TESTED	MONITORING FREQUENCY
Influent	Continuous		Flow	Totalized Daily
Influent	24 Hr Composite (flow		Biochemical Oxygen Demand Total Suspended Solids	Daily
Effluent	24 Hr Composite (flow Proportional)		Biochemical Oxygen Demand Total Suspended Solids Ammonia Nitrogen Total Phosphorous	Daily
Effluent	Grab		Dissolved Oxygen pH Chlorine Residual	Daily
Effluent	Grab		FecalColiform	Twice Weekly

Process Control Monitoring

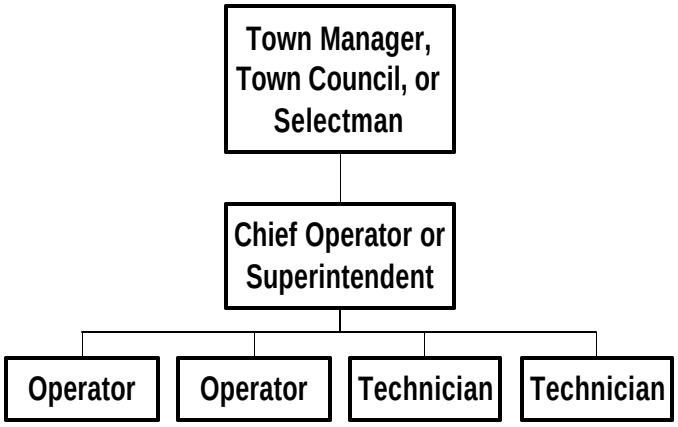
SAMPLE LOCATION	SAMPLE TYPE	SCHEMATIC REFERENCE	PARAMETERS TESTED	MONITORING FREQUENCY
Aeration Tank	Outlet Grab		Settleability (30 Min) Total Suspended Solids Volatile Solids	Daily
Aeration Tank	Contents in-place		Dissolved Oxygen	Continuous
Solids Concentrator	Product-grab		Percent solids	As Needed
Solids Concentrator	Decant-grab		Biochemical Oxygen Demand Total Suspended Solids Ammonia Nitrogen	As Needed
Digester Contents	Grab		Settleable Solids (30 Min.) Percent Solids Total Suspended Solids Volatile Suspended Solids	Daily
Clarifier	Grab		Blanket Depth Total Suspended Solids	Daily
Return Sludge	Grab		Total Suspended Solids	Daily
Filter Backwash	Grab		Biochemical Oxygen Demand Total Suspended Solids	As Neded

Attachment 3

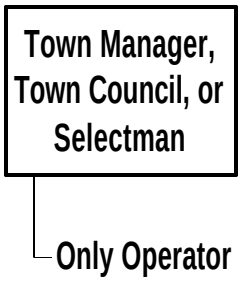
Maine DEP Form 49
Monthly Report of Operation

Attachment 4
Organizational Charts

Organizational Chart - Larger Facility



Organizational Chart - Smaller Facility



Attachment 5

Demonstration of Capability - Certification Statement

Date:	
Facility Name:	
Analyst(s):	
Matrix (circle):	Aqueous Soil Other:
Method # or SOP # :	

We, the undersigned, CERTIFY that:

1. The analyst(s) identified above, using the cited test method(s), which is in use at this facility for the analyses of samples, have met the Demonstration of Capability.

2. The test method(s) was performed by the analyst(s) identified on this certification.

3. A copy of the test method(s) and the laboratory-specific SOPs are available for all personnel on-site.

4. The data associated with the demonstration of capability are true, accurate, complete, and self-explanatory(*).

5. All raw data (including a copy of this certification form) necessary to reconstruct and validate these analyses have been retained at the facility, and that the associated information is well organized and available for review by authorized assessors.

6. The analyst(s) identified above, have read, understood, and agreed to perform the applicable (approved method or SOP as specified by laboratory management) revision of the method and/or SOP.

_____	_____	_____
Manager's or Trainer's Name	Signature	Date
_____	_____	_____
Analyst's Name	Signature	Date

* True: Consistent with supporting data
Accurate: Based on good laboratory practices consistent with sound scientific principles/practices.
Complete: Includes the results of all supporting performance data.
Self-Explanatory: Data properly labeled and stored so that the results are clear and require no additional explanation.

Attachment 6

SUMMARY OF DOCUMENTATION REQUIREMENTS

REQUIREMENT	STOCK STANDARDS LOGBOOK	STOCK STANDARDS LABEL	WORKING STANDARDS LOGBOOK	WORKING STANDARDS LABEL
Date of Receipt	X	X		
Supplier	X			
Description or Name of Standard	X		X	X
Lot Number of Standard	X			
Receiver's Initials	X			
Date Opened		X		
Date of Expiration	X	X	X	X
Date of Preparation			X	X
Initials of Preparer			X	
Components of Standard			X	
Unique Working Identification Number			X	X
Preparation Procedure			X	
Final Concentration			X	

Example Label

Date Received:
Date Opened:
Date of Expiration:

Facility Name

RECEIPT LOGBOOK FOR REAGENTS, STANDARDS, MEDIA & SOLVENTS

NAME - REAGENT, STANDARD, MEDIA OR SOLVENT?	DATE RECEIVED	MANUFACTURER	EXPIRATION DATE	LOT #	INIT.	DATE OPENED

REVIEWED BY: **DATE:**

Attachment 7

Facility Name

REFRIGERATOR or INCUBATOR TEMPERATURE LOG

Corrective Action: Note in the "comments" column and notify the QAO or supervisor; document corrective actions taken and return to control.

Thermometer Location				
Acceptance Criteria				
Thermometer ID				
Date	Initials	Temp (°C)	Temp (°C)	Comments

Attachment 8
Balance Calibration

Remove the working weights from the desiccator. Do not touch the weights with your hands. These weights should be handled only with the plastic forceps provided in the weights' case or with plastic gloves or KIM wipes. The small weights (i.e., $\leq$2 g) are often difficult to pick up with the forceps so use extra care when handling these weights.

Clean the balance pan and surrounding areas.

Check balance level (if applicable to the balance). If the air bubble is not centered in the circle of the level indicator, relevel the balance using the leveling screws. If necessary, ask for assistance.

Verify that the balance draft shield is in place or that the balance is free of air currents which could cause balance drift. Balances that are not equipped with draft shields must be carefully monitored to ensure they are free of air currents when in use.

Verify balance zero. Readjust or retare if necessary using procedures appropriate for the balance.

All verification activities must be recorded in a balance logbook. Each logbook should contain the following information:

- Balance Serial #
- Location of the balance
- Balance Manufacturer's Tolerance – this indicates the $\pm$ error of each reading
- Lowest weight to be weighed on the balance – this indicates the lowest weight that can be measured while still meeting the acceptance criteria for accuracy. Do not weigh anything on the balance below this weight. If lower weights need to be measured, another balance that meets the required calibration criteria at a lower weight range must be used.
- Date and initials of analyst
- True weights to be weighed and acceptance criteria for each weight
- Indication of pass/fail, comments and corrective actions

Using the weights appropriate (similar to the range being measured for the test), start by placing weight number one on the balance pan and recording its weight. Remove and be sure the weight returns to zero before proceeding with the remaining weights to be checked. Record all appropriate information in the logbook.

Based on the determined criteria, determine whether the measured weight passes or fails and indicate this in the logbook. If acceptance criteria are not met, do not continue to use the balance. Label the balance as "Out of Service" (initials and date) and immediately notify your manager. Document corrective actions taken, maintenance performed, and return to control in the logbook before resuming use of the balance.

FACILITY NAME
BALANCE CALIBRATION VERIFICATION LOG

Balance ID: **Serial #:**
Location:
Manufacturer's Tolerance: **Lowest Weight to be weighed on this balance:**
Corrective Action: If acceptance criteria is not met, do not continue to use the balance. Label the balance as "Out of Service" (initials/date) and notify your manager. Document corrective actions taken and return to control before using balance. Document any maintenance performed in the "Comments" section of the balance calibration verification log.

Date	Analyst Initials	Weight(gm) True Value	Weight(gm) Measured Value	Acceptance Criteria (gm)	Pass/Fail?	Comments – Corrective Actions and/or Maintenance

Attachment 9

Thermometer Verification

Thermometers must be calibrated annually at each temperature range that will be used. Upon receiving a NIST-certified thermometer, or equivalent from the manufacturer or from a company, which has calibrated the thermometer record the correct values and label the thermometer with the appropriate corrections by applying a label. To check other thermometer readings verses a calibrated thermometer it is necessary to check for corrections at the temperatures that the thermometer was calibrated at. Both thermometers must be kept immersed to the immersion line and in close contact in a solid medium such as sand or vermiculite for higher temperature checks that exceed the boiling point of water, or a liquid medium such as water. It is preferable to have the thermometers suspended and not touching the edges of the container since different materials conduct heat differently. If you are checking complete immersion thermometers the complete thermometer must be submerged. Place the container with both thermometers submerged to the immersion line and suspended in a medium into the refrigerator to check at 4° C, into the BOD incubator to check at 20°C, into the water bath to check at 44.5 °C into the oven to check at 103°C. Allow the thermometers and medium time to reach the appropriate temperature. Once the readings seem stable take readings on both thermometers and record. Allow the thermometers to remain at that temperature for another hour and record readings again to check for stability of readings. If the initial and final readings match, determine the difference if any from the calibrated thermometer and attach a label which indicates how much to adjust readings at each temperature checked. The amount of adjustment might vary from the low temperature range to the high temperature range. For instance at 4° C one might add 1° C whereas at 103° C one might subtract 0.5° C. Variation is dependent on the expansion of the metal or liquid component of the thermometer.

Attachment 10

FACILITY NAME – ADJUSTABLE PIPET CALIBRATION LOGBOOK

Pipet Serial Number _____ **Manufacturer**_____

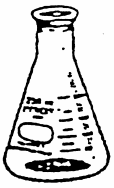
Pipet Range: **Min.**_____ **Mid.**_____ **Max.**_____

DATE	VOLUME CHECKED	H2O TEMP °C	VESSEL WEIGHT (GMS)	WEIGHT OF H2O DISPENSED OR REMOVED (GMS)			MEAN WGT / H2O DENSITY (mLs)	%CV	% DEV.	COMMENTS
				1	2	3				

Attachment 11

Example Glassware & Meniscus Reading

Beaker



Erlenmeyer Flask

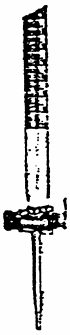
Roundbottom Flask

Buret

BOD Bottles

Graduated Cylinder

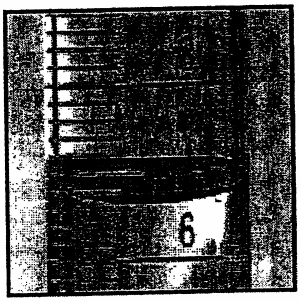
Test Tube



Volumetric Flasks

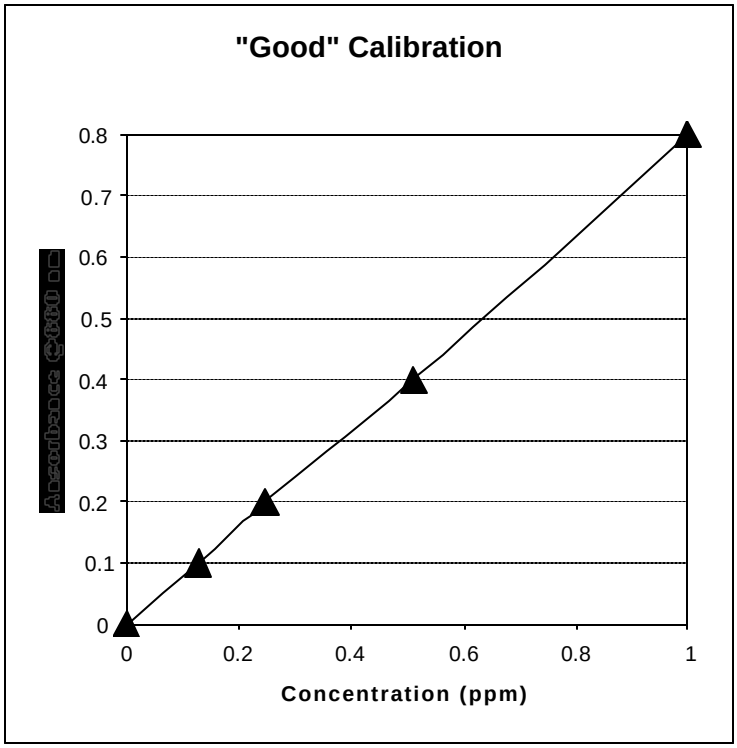
Pipets

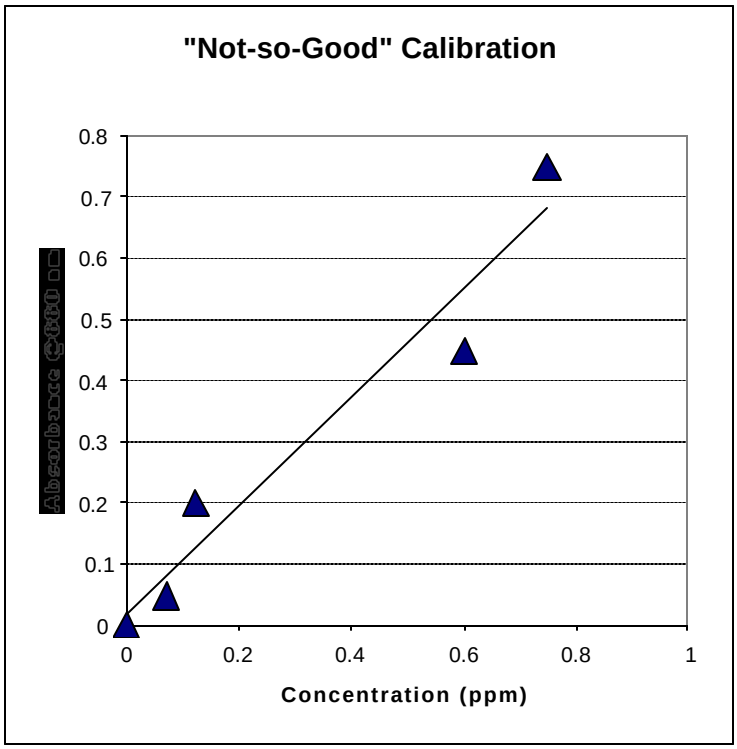
meniscus



Attachment 12

Example Calibration Curves





Attachment 13
Chain-of-Custody

Facility:	Contact:	Phone:	Fax:
Address:	City:	State:	Zip:
Sampler (Print/Sign):			

Notes:	Tests to be Performed & Preservative Used

Sample Description (Sample Identification and/or Lot #)	Date/Time Collected	Matrix	No. of Cntrs.	T	Preservative Here	Test Here	Preservative Here	Test Here	Preservative Here	Test Here	Preservative Here	Test Here	Preservative Here	Test Here	Preservative Here

Relinquished By:	Date/Time:	Received By:	Relinquished By:	Date/Time:	Received By:

Attachment 14

Example MDL

MDL for BOD

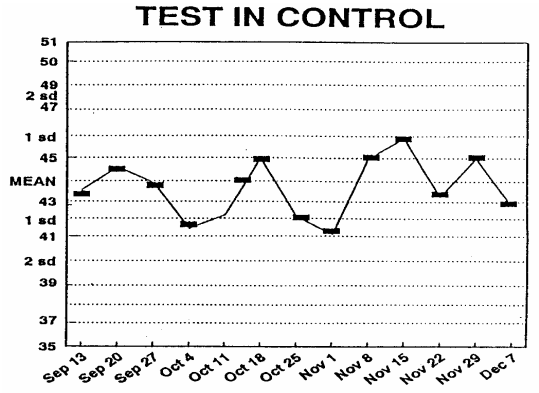
06/18/99

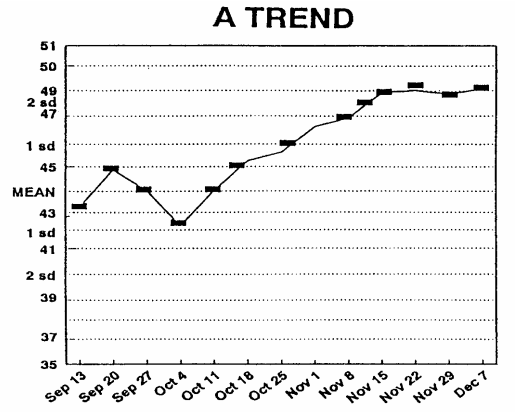
	Results	X-X	(x-x)
1	13.44	0.80	0.6346778
2	12.96	0.32	0.1002778
3	13.41	0.77	0.5877778
4	13.14	0.50	0.2466778
5	13.29	0.65	0.4181778
6	12.30	-0.34	0.1178778
7	12.69	0.05	0.0021778
8	10.17	-2.47	6.1173778
9	12.39	-0.25	0.0641778
10			
11			
Average	12.64		
sum/n-1			8.2892
n-1:			8
sum/n-1			1.03615
standard deviation:			1.01791
MDL:			2.9

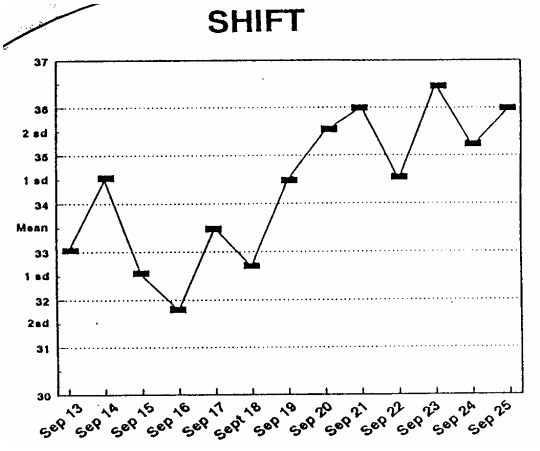
Number of Replicates	Degrees of freedom (n-1)	t, 0.99
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	15	2.602
21	20	2.528
26	25	2.485
31	30	2.457
61	60	2.39
0	0	2.326

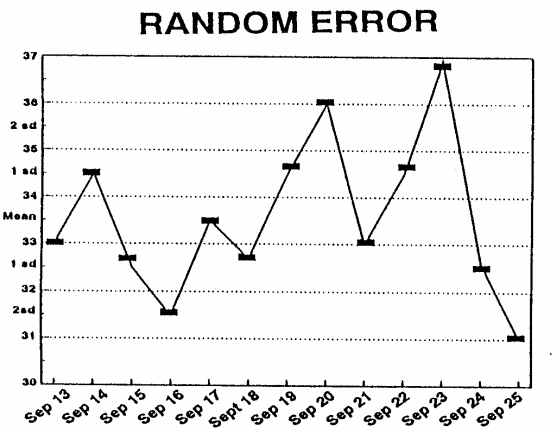
Attachment 15

Example Control Charts









Attachment 16

FACILITY NAME
MAINTENANCE LOG

DATE	INIT.	INSTRUMENT	PROBLEM OR PREVENTATIVE?	MAINTENANCE	DATE RETURN TO CONTROL

Please explain in detail what the problem is or if it is routine maintenance. Describe in detail the maintenance performed.

Attachment 17

Example Bench Sheets

Total Suspended Solids Analysis (mg/L)

Sample collection date: _____ time: _____ by: _____

Sample Location: ☐ Influent ☐ Effluent ☐ Other _____

Analysis run date: _____ time: _____ by: _____

Oven Temperature - In: _____ Out: _____

Drying Time - Initial: _____ Final: _____

Method used: _____

	Sample #1	**Sample #2**	**Sample #3**
weight of filter + dried solids (g)			
subtract tare weight of filter (g)			
weight of suspended solids (g)			
× 1,000 mg/g			
divided by sample volume (mL)			
× 1,000 mL/L			

Comments:

pH QUALITY CONTROL
CALIBRATION RECORD

Month of _____

Date	Cal.Std. 4.00	Cal. Std. 10.00	Check 7.00	Reading	Slope	Sample Temp.	Initials	Time
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								

BOD/CBOD BENCH SHEET

Sample Date: _____ Analysis Date: _____ Read Date: _____

Time: _____ Time: _____ Time: _____

Initials: _____ Initials: _____ initials _____

Incubator Temperature: Day 1: _____ Day 4: _____

Day 2: _____ Day 5: _____

Day 3: _____

Dechlorination: _____

Sample Type	Blank	Seed Blank	Infl BOD No. 1	Infl BOD No. 2	Effl. BOD No. 1	Effl BOD No. 2	G & G
Bottle #							
mL Sample							
Sample Temp.							
Initial DO							
Final DO							
Depletion							
Seed Correction							
BOD (CBOD) mg/L							

Calculations:

$$\text{BOD of Seed} = \text{DO Depletion} \times \frac{\text{Volume of Bottle}}{\text{mL of seed added to bottle}}$$

$$\text{Seed Correction} = \text{BOD of Seed} \times \frac{\text{volume of seed added to dilution bottle}}{\text{volume of bottle}}$$

$$\text{BOD of Sample (mg/L)} = \frac{(\text{DO Depletion} - \text{Seed Correction}) \times \text{volume of bottle}}{\text{mL of sample}}$$

MPN Test Data Sheet

Sample Date: _____ Analysis Date: _____

Sample Time: _____ Analysis Time: _____

Sample Number: _____ MPN/100 mL: _____

Location: _____

Initials of Sampler: _____ Initials of Analyst: _____

			Presumptive Test	Confirmed Test	
Volume mL.	Tube No.	24 hr	48 hr	24 hr	Comments
	1a				
	1b				
	1c				
	1d				
	1e				
	2a				
	2b				
	2c				
	2d				
	2e				
	3a				
	3b				
	3c				
	3d				
	3e				
	4a				
	4b				
	4c				
	4d				
	4e				
	5a				
	5b				
	5c				
	5d				
	5e				

Membrane Filter Test Sheet

Sample Date: _____ Analysis Date: _____

Sample Time: _____ Analysis Time: _____

Sample Number: _____ Selected Filter: _____

Initials of Sampler: _____ Initials of Analyst: _____

Location: _____ Colonies/100 mL: _____

Dish Number	Sample Volume mL	Colony Count

Quality Control

Fecal Coliform MFC Broth Preparation

Name of Media: _____ Lot #: _____

Expiration Date: _____

Date of Preparation: _____

Amount weighted: _____ gm Vol. Prepared: _____ mL

Sterilization by: _____

Prepared by: _____

Sterility Checked by: _____ Date: _____

Number of Colonies: _____

Growth Checked by: _____ Date: _____

Number of Colonies: _____

Phosphate Analysis

Sample Date: _____ Analysis Date: _____

Sample Time: _____ Analysis Time: _____

Sample Preserved? ☐Yes ☐ No Preservative: _____

Initials of Sampler: _____ Initials of Analyst: _____

Location: _____ Concentration mg/L: _____

Sample Type	Initial Volume	Final Volume	Absorbance	Concentration	Corrected Concentration

Attachment 18

FACILITY NAME– CORRECTIVE ACTION REPORT

Problem Identification (Person discovering problem) Name:			Date:
Affected Samples:			
LCS Failure	Prep Error	Transcription Error	Sample Contamination
Blank Contamination	Hold Time Missed	Detection Limit	Other
Poor Precision	Linearity	Calculation Error	Matrix Spike Failure
Details:			
Corrective Action Plan **Date:**			**Name:**
Details of Corrective Action Plan – Short Term and Long Term (if applicable):			
Review & Comments			
Chief Operator/Superintendent Approval:			Date:
Root Cause Investigation **(To be completed by handling QA Activities)**			
Lack of Organization	Lack of Training	Circumstances Beyond Control	Undetermined
Lack of Resources	Lack of Time	Lack of Top Management Support	Further Monitoring Needed
Lack of Experience	Lack of Discipline	Lack of Communication	Isolated Event
QA Approval:			Date:

LABORATORY

MANUAL

Revised May, 2004

Maine Wastewater Control Association

Dedicated to the Memory of

Susan Romatzick

(1956 – 1994)

PREFACE

The Maine WasteWater Control Association Laboratory Committee has revised the Association's Laboratory Manual. Procedures for chlorine residual by amperometric titration and for fecal coliform by MPN have been removed while procedures for *E. coli* by Colisure® and total phosphorus by block digestion and ascorbic acid have been added. More specific quality control procedures have also been added.

The Committee has chosen procedures it believes are most commonly used by wastewater labs in Maine. You can consult the Code of Federal Regulations 40CFRPart 136 for a complete list of approved methods for wastewater analytes. You should be aware that occasionally the EPA discontinues approval of old methods or the DEP may add requirements to a method. The Committee will work to make Association members aware of such changes, but individual permittees are ultimately responsible for compliance data submitted to a regulatory authority.

The Committee has been as meticulous as possible in preparing this manual. However, in the case of any errors, as stated above, individual permittees are solely responsible for their data.

Thanks are extended to the members of the Committee for researching, writing, and editing the procedures. It was an excellent educational opportunity for all of us.

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Mary Anne Peck	OMI Biddeford	
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Thanks are also extended to Don Albert, Maine DEP, and Matthew Sica, Lab Certification Officer Maine Bureau of Health, for their most helpful contributions.

If you have questions on anything in the lab manual, please contact the chair or any member of the Committee. Our goal is the production of accurate and reliable lab data across the State of Maine.

CONTENTS

Residue, Non-Filterable (Total Suspended Solids)…… EPA Method 160.2

Settleable Solids……Std. Method # 2540-F

Temperature…….. Std. Method # 2550-B

Total Chlorine Residual DPD Colorimetric……Std. Method # 4500-Cl-G

pH Analysis…….. Std. Method # 4500-H+B

Total Phosphorus…….. Hach Method 8190

Biochemical Oxygen Demand (BOD_5)…… Std. Method # 5210-B

Escherichia coli Membrane Filter Procedure……..……. Std. Method # 9213-D

Escherichia coli Colisure® Procedure……..…….. Std. Method # 9223 and Idexx

Fecal Coliform Membrane Filter Procedure……..…… Std. Method # 9222-D

Name of Facility
Laboratory SOP

Residue, Non-Filterable
(Total Suspended Solids)

EPA Method 160.2

Accepted by MWWCA Lab Committee Date May 12, 2004_

Revised by _____ Date _____

Revised by _____ Date _____

Revised by _____ Date _____

Residue, Non-Filterable (Total Suspended Solids)
EPA Method 160.2 (Gravimetric, Dried at 103-105°C

Sample Type: 24-Hour Flow-Paced Composite or Grab

Container: Glass or Plastic (Polyethylene or equivalent)

Sample Handling and Preservative:

Non-representative particulates such as leaves, sticks, fish and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result.

Preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to 4°C, to minimize microbiological decomposition of solids, is recommended.

Interferences:

Filtration apparatus, filter material, pre-washing, post-washing, and drying temperature are specified because these variables have been shown to affect the results.

Samples high in filterable residue (dissolved solids), such as saline waters, brines and some wastes, may be subject to a positive interference. Care must be taken in selecting the filtering apparatus so that washing of the filter and any dissolved solids in the filter minimizes this potential interference.

Equipment:

Analytical Balance - capable of weighing to 0.1 mg.

Desiccator – preferably containing a desiccant that utilizes a color indicator for moisture content.

Drying Oven - capable of maintaining a constant temperature between 103 - 105 ^{0}C.

Glass Fiber Filter Disks - these filters should not contain organic binder. For manufacturers of acceptable filters see page 2-56 of Standard Methods, 18th ed., 1992.

Filtration Apparatus - either a membrane filter funnel, Gooch crucible (25 to 40 ml capacity) or filter apparatus that contains a reservoir and a coarse (40 to 60 um) fritted disk as a filter support.

Filtration Setup - consisting of the filtration apparatus, side arm flask and vacuum source.

Glassware - it is recommended that all graduated cylinders used to measure the sample volume be of Class A quality. If pipettes are used to measure sample volumes, wide tip serological pipettes should be utilized.

Planchet - filters while drying are placed on these small aluminum or stainless steel disks, are used. (Planchets are not needed when using Gooch crucibles or when the filters are placed in platinum crucibles.)

Reagents: None.

Procedure:

1. Preparation of glass fiber filter disc: Place the glass fiber filter on the membrane filter apparatus or insert into bottom of a suitable Gooch crucible with wrinkled side up. While vacuum is applied, wash the disc with three successive 20-ml volumes of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Remove filter from membrane filter apparatus or both crucible and filter if Gooch crucible is used, and dry in an oven at 103-105 degrees C for one hour. Remove to desiccator and store until needed. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg). Weigh immediately before use. After weighting, handle the filter or crucible/filter with forceps or tongs only.

2. Selection of Sample Volume: For a 4.7 cm diameter filter, filter 100 ml of sample. If weight of captured residue is less than 1.0 mg, the sample volume must be increased to provide a least 1.0 mg of residue. If other filter diameters are used, start with a sample volume equal to 7 ml/cm and collect at least a weight of residue proportional to the 1.0 mg stated above.

3. Note: If during filtration of this initial volume the filtration rate drops rapidly and filtration time exceeds 5 to 10 minutes, the following scheme is recommended:
   Place an unweighed glass fiber filter of choice affixed in the filter assembly. Add a known volume of sample to filter funnel and record the time elapsed after selected volumes have passed through the filter. Twenty-five ml increments for timing are suggested. Continue to record the time and volume increments until filtration rate drops rapidly. Add additional sample if the filter funnel volume is inadequate to reach a reduced rate. Plot the observed time versus volume filtered. Select the proper filtration volume as that point short of the time a significant change in filtration rate occurred.

4. Prior to filtering sample, the sample should be brought up to room temperature.

5. Assemble the filtering apparatus and begin suction. Wet the filter with a small volume of distilled water to seat it against the fritted support.

6. Shake the sample vigorously and quantitatively transfer the predetermined sample volume to the filter using a graduated cylinder. Remove all traces of water by continuing to apply vacuum after the sample has passed through. With suction on, wash the graduated cylinder, filter, non-filterable residue (suspended solids), and funnel wall with three portions of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Note: total volume of wash water used should equal approximately 2 ml per cm. Using 4.7 cm filter the total volume is 30 ml.

7. Carefully remove the filter from the filter support. Alternatively, remove crucible and filter from crucible adapter. Dry at least one hour at 103-105 degrees C. Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained (weight difference is less than 0.5 mg).

   Note: In order for data to be acceptable, the oven must remain within the 103-105 degree C limit for the entire drying cycle.

Temperature Monitoring:

The temperature of the drying oven should be monitored at least once per day during normal working hours, whenever compliance monitoring samples are being dried in the oven. The reading of the oven temperature should be accomplished without having to open the oven door.

Thermometers should be calibrated annually against a National Bureau of Standards traceable thermometer and then documented as to date, time and persons performing the calibration.

Troubleshooting: See Procedure and Interferences sections above.

Calculation: TSS (mg/l)= $\frac{(A - B) * 1000}{\text{Sample vol. (mls)}}$

A = weight of filter + dried residue in mg.
B = weight of filter in mg.

Remember to correctly use this formula one must convert the balance readings from g to mg.

Precision and Accuracy:

With regard to precision, ten percent duplicate or replicate samples should be run. This would result in on duplicate sample being run every ten samples.

A Replicated Sample is defined as a sample that comes from the same bottle and is split in the laboratory and each split sample is analyzed at the same time.

A Duplicate Sample is defined as a sample that is taken at the same time but collected in the field in a different bottle. One example of how duplicate samples can be reasonably collected is manually filling two individual bottles at the same time.

Quality Control (QC):

Equipment:
Drying oven temperature should be checked and recorded at least once per day whenever compliance monitoring samples are being dried in the oven.
Yearly calibration checks on analytical balance should be performed along with monthly balance checks using standard weights.
Desiccant used in the desiccator should be changed as needed. It is recommended that a color-indicating desiccant be utilized.

Replicate Analysis:
At a minimum frequency of 10%, compliance samples should analyzed in replicate. Precision of analysis should be +/- 10% calculated as the difference as a percentage of the mean. $(100(x_1-x_2)/\text{mean } x)$

Externally Supplied Standards:
At least once per year an externally supplied standard should be analyzed.

Note: This procedure has been excerpted in its entirety from EPA Method 160.2 (Gravimetric, Dried at 103-105° C) from "Methods for Chemical Analysis of Water and Wastes" (EPA-600/4-79/020). This information is meant only to summarize and not be a replacement for these references.

Name of Facility
Laboratory SOP

Settleable Solids
Standard Methods 2540-F

Accepted by MWWCA Lab Committee Date May 12, 2004_

Revised by _____ Date _____

Revised by _____ Date _____

Revised by _____ Date _____

Settleable Solids Std. Method # 2540-F*

Sample Type: Grab

Container: Glass or Plastic (Polyethylene or Equivalent)

Preservative: Refrigerate at 4ºC and analyze within 48 hours.

Interferences: None reported.

Equipment:

Imhoff Cone

Stirring rod – long enough to reach near bottom of the cone

Timer – must measure at least 1 hour in 45 minute and 15 minute intervals

Reagents: None.

Procedure:

1. *Fill a clean Imhoff cone to the 1 Liter mark with a well-mixed sample.*

2. Allow solids in sample to settle for 45 minutes.

3. After 45 minutes, gently stir the sides of the Imhoff cone with a stirring rod, being careful not to disturb the solids that have already settled. Rather than using a rod, you can also gently rotate Imhoff cone to loosen any solids attached to the inside wall.

4. Allow solids to settle for an additional 15 minutes. Read and record the volume of settleable solids in the Imhoff cone as milliliters per liter.

Troubleshooting:

Occasionally when analyzing influent or other such samples for settleable solids, there may be a few pockets of liquid between the large settled particles. The volume of these liquid pockets may be subtracted from the total volume of the settleable solids, but only when the volumes of the individual liquid pockets are greater than 0.1 mL. When a separation of settleable and floatable materials occurs, do not estimate nor include the volume of floatable material as settleable solids.

Precision and Accuracy:

The practical lower limit of measurement depends on the cleanliness of the Imhoff Cones and the clarity of the water being analyzed. Generally the limit of detection is 0.1 mL/L for effluent samples and may be higher for other wastewater samples.

Note: From Standard Methods for the Examination of Water and Wastewater, 18th Edition. Copyright 1992 by the American Public Health Association, the American Water Works Association and the Water Environment Federation. Reprinted with permission. This information is meant only to summarize and not be a replacement for these references.

Name of Facility
Laboratory SOP

Temperature
Standard Methods 2550-B

Accepted by MWWCA Lab Committee Date May 12, 2004_

Revised by _____ Date _____

Revised by _____ Date _____

Revised by _____ Date _____

Temperature Std. Method # 2550-B

Sample Type: Grab

Container: Glass or Plastic. (Polyethylene or Equivalent)

Preservative: None. Analyze within 15 minutes.

Interferences: None reported.

Equipment:

Thermometer, mercury or spirit filled. Use thermometers graduated to 0.5ºC to monitor most incubators and refrigerators. Use thermometers graduated to 0.1ºC for incubators operated above 40ºC.

Reagents: None

Procedure:

When obtaining temperature measurements, the thermometer should be immersed up to the immersion line on a partial immersion thermometer or totally immersed in the sample when using a total immersion thermometer. Check for those markings on the thermometer prior to use.

Precision and Accuracy:

Procedure to verify accuracy by comparison with a NIST-certified thermometer, or equivalent. It must be performed on an annual basis.

1. Upon receiving a NIST-certified thermometer, or equivalent from the manufacturer or from a company which has calibrated the thermometer, record the correct values and label the thermometer with the appropriate corrections by applying a label.

2. To check other thermometer readings versus a calibrated thermometer it is necessary to check for corrections at the temperatures that the thermometer was calibrated at. Both thermometers must be kept immersed to the immersion line and in close contact in a solid medium such as sand or vermiculite for higher temperature checks that exceed the boiling point of water, or a liquid medium such as water. It is preferable to have the thermometers suspended and not touching the edges of the container since different materials conduct heat differently. If you are checking complete immersion thermometers the complete thermometer must be submerged.

3. Place the container with both thermometers submerged to the immersion line and suspended in a medium into the refrigerator to check at 4^0 C, into the BOD incubator to check at 20^0C, into the water bath to check at 44.5 ^{0}C into the oven to check at 103^0C. Allow the thermometers and medium time to reach the appropriate temperature. Once the readings seem stable take readings on both thermometers and record.

4. Allow the thermometers to remain at that temperature for another hour and record readings again to check for stability of readings. If the initial and final readings match, determine the difference if any from the calibrated thermometer and attach a label which indicates how much to adjust readings at each temperature checked. The amount of adjustment might vary from the low temperature range to the high temperature range. For instance at 4^0 C one might add 1^0 C whereas at 103^0 C one might subtract 0.5^0 C. Variation is dependent on the expansion of the metal or liquid component of the thermometer.

Note: From Standard Methods for the Examination of Water and Wastewater, 18th Edition. Copyright 1992 by the American Public Health Association, the American Water Works Association and the Water Environment Federation. Reprinted with permission. This information is meant only to summarize and not be a replacement for these references.

Name of Facility
Laboratory SOP

Total Chlorine Residual by DPD
Standard Methods 4500-Cl G

Accepted by MWWCA Lab Committee Date May 12, 2004_

Revised by _____ Date _____

Revised by _____ Date _____

Revised by _____ Date _____

Total Chlorine Residual Std. Method # 4500-Cl-G*
DPD Colorimetric Method

Sample Type: Grab

Container: Glass or Plastic (Polyethylene or equivalent)

Preservative: Analyze Immediately

Interferences:

Compensate for color and turbidity by using sample to zero the photometer. If chromate is present in the sample, its interference can be minimized by using the thioacetamide blank correction. (See page 4-46 of Standard Methods, 18th ed., 1992.

Equipment:

Photometric equipment - consisting of either a spectrophotometer using a wavelength of 515 nm and providing a light path of 1 cm or longer, or a filter photometer equipped with a filter having a maximum transmission in the wavelength range of 490 to 530 nm and providing a light path of 1 cm or longer.

The HACH DR 100 and other similar equipment meet the above criteria and therefore can be used for measurement of chlorine residuals ³ 0.1 mg/L.

Glassware - Use separate glassware, including separate spectrophotometer cells, for free and combined chlorine measurements. This will avoid iodide contamination when measuring samples for free chlorine.

When measuring total residual chlorine levels below 0.1 mg/l, the light path length must be between 5 and 10 cm.

Reagents:

Phosphate Buffer Solution: Dissolve 245 g anhydrous sodium phosphate dibasic (Na_2HPO_4) and 46 g anhydrous potassium phosphate monobasic (KH_2PO_4) in distilled water. Combine with 100 ml distilled water in which 800 mg disodium ethylenediamine tetraacetate dihydrate, (EDTA), have been dissolved. Dilute to 1 liter with distilled water and add 20 mg mercuric chloride ($HgCl_2$) to prevent mold growth and interference in the free chlorine test caused by any trace amounts of iodide in the reagents. (**Caution: $HgCl_2$ is toxic - take care to avoid ingestion.**)

N,N-Diethyl-p-phenylenediamine (DPD) Indicator Solution: Dissolve 1 g DPD oxalate, or 1.5 g DPD sulfate pentahydrate, or 1.1 g anhydrous DPD sulfate in

chlorine-free distilled water containing 8 ml of 1 + 3 sulfuric acid (H_2SO_4) and 200 mg disodium EDTA. Make up to 1 liter, store in a brown, glass-stoppered bottle in the dark, and discard when discolored. Periodically check solution blank for absorbance and discard when absorbance at 515 nm exceeds 0.002/cm.

N,N-Diethyl-p-phenylenediamine (DPD) Indicator Pillows: Pre-mixed DPD reagent pillows such as those available from the Hach Co. are an acceptable substitute for the DPD indicator solution provided it can be verified that the composition of the powder pillows matches the reagents required by an approved method.

Standard Ferrous Ammonium Sulfate (FAS) Titrant: Dissolve 1.106 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water containing 1 ml 1 + 3 H_2SO_4 and cooled distilled water. This standard may be used for 1 month, and then the titer must be checked against potassium dichromate.

Potassium Iodide: Crystals

Potassium Iodide Solution: Dissolve 500 mg KI and dilute to 100 ml, using freshly boiled and cooled distilled water. Store in a brown, glass-stoppered bottle preferably in a refrigerator. Discard when the solution becomes yellow.

Sodium Arsenite Solution: Dissolve 5.0 g $NaAsO_2$ in distilled water and dilute to 1 liter. (**Caution: Toxic - Take care to avoid ingestion**)

Thioacetamide Solution: Dissolve 250 mg CH_3CSNH_2 in 100 ml distilled water. (**Caution: Suspected Cancer Causing Agent. Take care to avoid ingestion.**)

Chlorine Demand-Free Water: Prepare by adding sufficient chlorine to a good quality distilled or deionized water to give 5 mg/l free chlorine residual. Let this stand for 2 days in the dark and then check residual. Remove remaining free chlorine by placing container in the light. For further details see Standard Methods, 18th or 19th edition, p 4-40.

Procedure:

1. Prior to use, the spectrophotometer must be first calibrated with either chlorine or potassium permanganate solutions. Be sure to use chlorine demand-free water and glassware to prepare your standards. See Standard Methods 18th edition, 1992, page 4-46 for the details for making up the calibration curve.

2. The HACH DR 100 and similar instruments already have an internal standard curve. This allows samples analyzed via this instrument to be directly read. However, it is good laboratory practice to routinely check the response of the instrument with a known chlorine residual standard.

3. Use a sample volume that is appropriate to the photometer or colorimeter. When the total chlorine residual exceeds 4 mg/L, the sample should be diluted with chlorine demand-free water.

4. The easiest way to add the DPD reagent is to use the commercially available "HACH DPD Total Chlorine Reagent Powder Pillows". These are usually suitable for samples up to 25 mls. After adding this reagent to the sample, swirl the reagent and sample together and allow 3 minutes but no more than 6 minutes for the reaction to occur. The absorbance or percent light transmittance can then be read at 515 nm.

Troubleshooting: See the Interference section above.

Calculation:

When using an instrument that does not already have an internal standard curve, a standard curve must be developed. This is accomplished by plotting the absorbance of the calibration standards against the chlorine equivalent concentrations and computing the sample concentration directly from the linear curve. Percent transmittance readings can be utilized in place of absorbance provided the graph paper is semi-log and the percent transmittance readings are placed on the log side of the graph paper. To plot the straightest line, a linear regression should be performed on the data.

Precision and Accuracy:

This method is one of the preferred methods recommended by US EPA Region I staff for the measurement of total chlorine residual measurements at levels $\geq$ 0.1 mg/L. However, this method can be used as an alternate method for the measurement of total residual chlorine at levels less than 0.1 mg/L provided a light path between 5 and 10 cm is utilized.

The recommended control limit for Total Chlorine Residual is one standard deviation. In establishing its control limits, it is recommended that initially each laboratory perform a minimum of 25 total residual chlorine measurements of standards over a period of several weeks or months and calculate the mean and the standard deviation. Once the control limits for this test are completed, then each laboratory should perform a minimum of 1 total chlorine measurement of a standard per every twenty samples.

With regard to precision, five percent duplicate and replicate samples should be run. This would result in one duplicate sample and one replicate sample being run every twenty samples.

A **Replicate Sample** is defined as a sample that comes from the same bottle and is split in the laboratory and each split sample is analyzed at the same time.

A **Duplicate Sample** is defined as a sample that is taken at the same time but collected in the field in a different bottle. One example of how duplicate samples can be reasonably collected is by running the sampler manually and filling two individual bottles at that time.

* - With the exception of the references to HACH reagents and equipment, this procedure has been excerpted from Standard Methods For The Examination Of Water And Wastewater, 18th edition. Copyright 1992 by the American Public Health Association, the American Water Works Association and the Water Environment Federation. Reprinted with permission. This information is meant only to summarize and not be a replacement for these references.

Name of Facility
Laboratory SOP

pH
Standard Methods 4500-H^+ B

Accepted by MWWCA Lab Committee Date May 12, 2004_

Revised by _____ Date _____

Revised by _____ Date _____

Revised by _____ Date _____

pH Analysis Std. Method # 4500-H+B.

Sample Type: Grab

Container: Glass or Plastic (Polyethylene or Equivalent)

Handling and Preservation:

None. Analyze immediately or within 15 minutes of sampling.

High-purity waters and waters not at equilibrium with the atmosphere are subject to changes when exposed to the atmosphere, therefore the sample containers should be filled completely and kept sealed prior to analysis.

Interferences:

The glass electrode, in general, is not subject to solution interferences from color, turbidity, colloidal matter, oxidants, reductants or high salinity.
Sodium error at pH levels greater than 10 can be reduced or eliminated by using a "low sodium error" electrode.

Coatings of oily material or particulate matter can impair electrode response. Gentle wiping or detergent washing, followed by distilled water rinsing can usually remove the coatings. An additional treatment with hydrochloric acid (1 + 9) may be necessary to remove any remaining film.

Temperature effects on the electrometric measurement of pH arise from two sources. The first is caused by the change in electrode output at various temperatures. This interference can be controlled with instruments having temperature compensation or by calibrating the electrode-instruments system at the temperature of the samples. The second source is the change of pH inherent in the sample at various temperatures. This error is sample dependent and cannot be controlled; it should therefore be noted by reporting both the pH and temperature at the time of analysis.

Equipment:

pH meter - consisting of potentiometer, a combination pH electrode, and a temperature-compensating device. The pH meter should be capable of performing at least a 2 buffer calibration. Also, the meter should be capable of reading to 0.01 standard units and successive readings should be accurate and reproducible to 0.1 standard units and have a range of 0 to 14.

Magnetic stirrer - with magnetic stirring bar.

Container for buffers and samples - with sufficient room to cover sensing elements of the electrode and give adequate clearance for the magnetic stirring bar.

Distilled water container - to rinse off probe between samples.

Magnetic rod - to remove stirring bar.

Soft tissue - to blot off excess water on probe.

Reagents: Buffer Solutions - Utilize either commercially available standardized color-coded pH buffers (preferred) or to prepare buffers fresh, see Standard Methods 18th edition, 1992, pp 4-67 to 4-68.

Procedure:

1. pH meters should be calibrated each day or each shift. When pH measurements are taken more than once a day, then the calibration of the pH meter should be verified by checking it against at least one buffer prior to each use.

2. Follow the manufacturer's instructions for calibrating a particular pH meter and probe or use the following: Remember to always document each time the pH meter is calibrated.

3. *Before being placed in the initial buffer, the electrodes should be removed from their storage solution, rinsed with distilled water and blotted dry with a soft tissue. This procedure of rinsing the electrode and blotting it dry should be followed whenever changing the electrodes from one solution to another, regardless of whether the change is to another buffer or sample.*

4. Use two or three buffers, depending on the type of meter you are calibrating, to bracket any samples being analyzed for pH. One of the buffers should have a pH of 7. The pH 7 buffer is used first to set the response point for the probe. The other buffers are then used to set the slope of the pH probe's response.

5. Once the pH meter has been calibrated, check its accuracy by using another buffer from a different stock number. This buffer should be within 0.1 pH units of its actual value. If the difference is greater than 0.1 pH units from the expected value then look for trouble with the electrodes or the meter.

6. Buffers and samples should be at the same temperature. When changing the temperature of a buffer, be sure to calibrate meter for the correct pH value for the temperature of the buffer. In addition, buffers should be used before the label's expiration date or within one year from when use is initiated. If growth is observed in a bottle of buffer discard the buffer. The individual portions of buffers being used during a calibration and analyses should be discarded after each batch of samples is analyzed. They should never be returned to the original bottles of buffer. Finally, if pH 12 buffer is being used, a pH preservative needs to be added to the buffer.

7. During analysis of pH, samples and buffers should be stirred continuously and gently so that carbon dioxide entrainment in the solution is minimized.

8. For buffered samples or those of high ionic strength, the electrodes should be conditioned after cleaning by dipping them into the sample for one minute. The electrode should then be blotted dry and immersed in a fresh portion of the same sample. The pH for this type of sample can then be read.

9. For dilute, poorly buffered solutions, immersing them in three or four successive portions of the sample should equilibrate the electrodes. Then a fresh sample should be used to measure pH.

Troubleshooting:

Potentiometer**:** Consult manufacturer's instructions for troubleshooting.

Electrodes**:** If potentiometer is functioning properly, look for the instrument fault in the combination electrode. Glass electrodes fail because of scratches, deterioration, or accumulation of debris on the glass surface. Rejuvenate electrode by alternately immersing it three times each in 0.1 N hydrochloric acid (HCl) and 0.1N sodium hydroxide (NaOH). After rejuvenation, soak in pH 7.0 buffers or commercial electrode storage solution overnight. Rinse and store in pH 7.0 buffer. Rinse again with distilled water before use. Protein coatings can be removed by soaking glass electrodes in a 10% pepsin solution adjusted to pH 1 to 2.

Reference electrode troubles generally are traceable to a clogged junction. Interruption of the continuous trickle of electrolyte through the junction causes increase in response time and drift in reading. Clear a clogged junction by applying suction to the tip or by boiling tip in distilled water until the electrolyte flows freely when suction is applied to tip or pressure is applied to the fill hole. Replaceable junctions are available commercially.

Precision and Accuracy:

When pH meter and electrodes are in good working condition and proper procedures are consistently utilized, a precision of +/- 0.02 pH units and an accuracy of +/- 0.05 pH units can be achieved. However, under normal conditions, the limit of accuracy is expected to be +/- 0.1 pH units. This is especially true when water samples or poorly buffered solutions are analyzed for pH.

Note: This procedure has been excerpted in its entirety from Standard Methods for the Examination of Water and Wastewater, 18th Edition, Copyright 1992 by the American Public Health Association, the American Water Works Association and the Water Environment Federation, Reprinted with permission; and from EPA Method 150.1 (pH Electrometric) from "Methods for Chemical Analysis of Water and Wastes" (EPA-600/4-79/020). This information is meant only to summarize and not be a replacement for these references.

Name of Facility
Laboratory SOP

Biochemical Oxygen Demand (BOD_5)

9.3Standard Methods 5210-B

Accepted by MWWCA Lab Committee Date May 12, 2004

Revised by _____ Date _____

Revised by _____ Date _____

Revised by _____ Date _____

Biochemical Oxygen Demand (BOD_5) Std. Method # 5210-B*

Sample Type: Flow- or Time-paced composites or Grab samples

Container: Glass or Polyethylene

Preservative:

Grab samples: If analysis is begun within 2 hours of sample collection, cooling to 4 ^{o}C is unnecessary. If sample is held longer than 2 hours then keep the sample between 1 to 4 ^{o}C from time of collection until analysis. Analysis within 6 hours of collection is preferable but in no case should analysis begin more than 24 hours after collection of sample.

Composite samples: Keep samples between 1 to 4 ^{o}C during compositing. Limit compositing period to 24 hours. If analysis is begun within 2 hours of completing composite sample collection, continued cooling at 4 ^{o}C is unnecessary. If composite sample is to be held longer than 2 hours, then keep sample between 1 to 4 ^{o}C from the time sample collection is completed until analysis. Analysis within six hours of completing compositing is preferable but in no case should analysis begin more than 24 hours after sample collection.

Interferences:

Samplers, sample containers and sample lines must be clean to minimize degradation of the sample during the compositing period. In addition, all glassware should be free of both air-borne dust and dirt and bacteriostatic (bacteria killing) agents.

If dilution water is of poor quality, or if the reagents are contaminated, the dilution water will cause the BOD to be higher than actual and may result in “bad” BOD blanks.

Inaccurate results occur if the oxygen depletion in the dilution water blanks exceed 0.2 mg/L, therefore, the maximum depletion allowed for dilution water is $\pm$ 0.2 mg/L.

Sample pH should be within the range of 6.5 to 7.5 standard units. If samples are neutralized, then samples need to be seeded. When neutralizing samples, a solution of either sulfuric acid or sodium hydroxide can be utilized. The strength of these solutions should be such that they do not dilute the BOD sample by more than 0.5 %. Acid and base strengths of 1 Normal are usually sufficient.

Samples that contain residual chlorine must be dechlorinated at the time of collection to prevent inhibition of bacteria activity. In addition, these samples and other samples disinfected by any other means must be seeded.

Samples containing other toxic materials such as heavy metals need to be seeded and evaluated for "sliding scale" BOD. "Sliding scale BOD's" are the result of toxicity present in a sample. The toxin(s) in the sample kill off or impair the ability of bacteria to oxidize the organic material in a sample. The greater the amount of sample present, the greater the toxic impact. As a consequence, the BOD dilutions with the smallest sample size wind up with the highest BOD values because they have the least amount of toxin present. And, as the sample size increases, the BOD of the sample decreases.

Inaccurate results may occur when samples have initial (Day 1) dissolved oxygen concentrations greater than (>) 9.1 mg/L.

Samples are incubated in the dark to prevent photosynthesis.

Inaccurate dissolved oxygen measurements caused by malfunctioning equipment or improper meter calibration can result in either an inaccurate increase or decrease in the test results.

Introduction of air into BOD bottles will invalidate the BOD results for those bottles. Air can be introduced into BOD bottles because of evaporation of the water seal; the trapping of air bubbles during sample setup; or incubating the BOD samples with initial (Day 1) dissolved oxygen levels that exceed 9.1 mg/L.

Loss of BOD due to volatilization of organics may be caused by excessive mixing, shaking, or stirring of the sample(s).

Equipment:

BOD Bottles - 300 mL capacity with ground glass stoppers and plastic sealing caps.

Air Incubator or Water Bath – Thermostatically controlled at 20 ± 1 Deg C and able to exclude all light.

Large Bottle or Carboy – Can be glass or polyethylene with a capacity of 1 to 5 gallons for use in storing dilution water. It is preferable to have this container fitted with a siphoning attachment.

Dissolved Oxygen Meter or Winkler Apparatus – See *Standard Methods*, 18th edition, 1992, pages 4-104 to 4-105 and 4-98 to 4-102.

Pipettes – Wide-bore (3mm) graduated or volumetric pipettes. When sample volume exceeds 25 mLs, the use of TD ("to deliver) graduated cylinders is recommended.

Bubbler – Source of bubbler equipment to fit dilution water storage bottle for aeration (Optional). This system must not blow oil or other contaminants into the dilution water.

Reagents:

Phosphate Buffer: Dissolve 8.5 g potassium dihydrogen phosphate (KH_2PO_4), 21.75 g potassium monohydrogen phosphate [dibasic] (K_2HPO_4), 33.4 g sodium monohydrogen phosphate [dibasic] ($Na_2HPO_4 \cdot 7H_2O$) and 1.7 g ammonium chloride (NH_4Cl) in approximately 500 mL distilled or deionized water and dilute to 1 L. The pH should be 7.2 without further adjustment. Note: Dissolving these reagents takes a considerable amount of time.

Magnesium Sulfate: Dissolve 22.5 g $MgSO_4$ $7H_2O$ in distilled or deionized water and dilute to 1 L.

Calcium Chloride: Dissolve 27.5 g $CaCl_2$ in distilled or deionized water and dilute to 1 L.

Ferric Chloride: Dissolve 0.25 g $FeCl_3$ $6H_2O$ in distilled or deionized water and dilute to 1 L. Note: This chemical will undergo a normal darkening of color over time.

Nutrient Pillows: Nutrient pillows for the BOD test are an acceptable substitute for the four nutrient solutions identified above, provided it can be verified that the composition of the powder pillows matches the reagents required by an approved method.

Glucose-glutamic acid solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103 ^{o}C for 1 hour. Add 150 mg glucose and 150 mg glutamic acid to distilled or deionized water and dilute to 1 L. Prepare fresh immediately prior to each use. Alternately, use premixed GGA solution purchased from a commercial supplier.

Acid and Alkaline Solutions: Use 1N Sulfuric Acid or 1N Sodium Hydroxide solution to neutralize caustic or acidic BOD samples.

Sodium Sulfite: Dissolve 1.575 g Na_2SO_3 in distilled or deionized water and dilute to 1 L. This solution is used to dechlorinate samples and is not stable so it must be prepared daily.

Cleaning:

All glass or polyethylene equipment used for the test should be washed with a biodegradable phosphate-free detergent using a bottle brush, then rinsed

exhaustively with hot tap water followed by distilled or distilled/deionized water rinsing 3 times. Place precleaned BOD bottles upside-down to air dry.

Sample Size:

Insure that an adequate volume of sample is on hand to run a minimum of 3 dilutions.

Practically all wastewater plants operate within a predictable BOD range for various sampling points based on previous in-house and NPDES analyses of record. These should be consulted prior to using this method.

Select the previous highest and lowest recorded BOD values from the above record for the types of samples desired, (influent, primary effluent, secondary effluent, etc.). Determine a reasonable BOD value from the record and consult Table 1.

The dilutions selected should show oxygen depletions ranging from 2 to 6 mg/L. Sample sizes may need to be varied periodically as required by the plant operating conditions or as judged by the TSS test results.

Dilution H_2O:

Before use, saturate the dilution water with dissolved oxygen by shaking in a partially filled bottle or by aerating with organic-free air. Alternatively, store dilution water in cotton-plugged or capped bottles long enough to become saturated with dissolved oxygen. If the dilution water is shaken or aerated to maximize the dissolved oxygen, it should be allowed to stand an absolute minimum of 15 minutes to remove pinpoint air bubbles. Allowing the water to stand for a longer period provided the opening is protected from contamination is acceptable.

The source of dilution water is not restricted and may be distilled, distilled/ deionized, tap, or receiving stream water free of biodegradable organics and bio-inhibitory substances such as chlorine or heavy metals.

Inaccurate results occur if the oxygen depletion in the nutrient water blanks exceeds 0.2 mg/L; therefore, the maximum depletion allowed for nutrient water is 0.2 mg/L.

Allow approximately 1 Liter of nutrient water per BOD sample.

If using nutrient powder pillows, follow the directions that accompany the nutrient pillows. Be sure that all the chemicals contained in the pillows are transferred to the dilution water.

If using lab-prepared nutrients, add 1 mL each of the phosphate buffer, magnesium sulfate, calcium chloride, and ferric chloride solutions per liter of dilution water. Rinse pipette with distilled deionized water between each addition of nutrients. When using the nutrient solutions, always pour the reagents out into small clean beakers and then pipette them into the dilution water. Never pour any liquid back into the flasks or dip a pipette into the larger stock nutrient bottles; otherwise, biological contamination will result. **Discard nutrient reagents if there is any sign of biological growth in the stock bottle(s) or Erlenmeyer flask(s).**

Seeding:

This technique is used when the bacteria that would normally be present in a BOD sample are either absent or may have been exposed to toxic substances such as heavy metals or pH values outside of the 6.5 to 7.5 range. Some examples of where the seeding of BOD samples is employed are: glucose-glutamic acid quality assurance samples; disinfected effluent samples; dechlorinated effluent samples; or industrially-influenced samples that may or may not exhibit signs of sliding scale BOD's (toxicity).

The best source of seed comes from a biological wastewater plant that is treating the type of waste being analyzed for BOD. Therefore, one day prior to setting up the BOD run, allow about 100 mLs of settled secondary effluent or MLSS to stand at room temperature. The “clear” supernatant can then be used as a source of seed bacteria.

Seed may be added directly to the BOD bottles or to the dilution water. The seed should have an oxygen demand ranging from 0.6 to 1.0 mg/L. To calculate the seed demand, the BOD of the seed must be analyzed at the same time the other BOD samples are run.

Set up/Procedure:

1. All samples and the dilution water should be brought to a temperature close to 20 Deg C.

2. Fill two or three BOD bottles with dilution water by siphoning or pouring without splashing or bubbling. These are the dilution water blanks.

3. Use three dilutions for each BOD sample. Pipette or pour the appropriate sample volume directly into each BOD bottle, seed if necessary and then fill the bottle up to the ground glass line in the neck of the BOD bottle. These transfers of liquid should be accomplished with the minimal amount of agitation.

4. Analyze all BOD sample dilutions and blanks for dissolved oxygen content. This is the initial (Day 1) dissolved oxygen reading. The initial dissolved oxygen

content of all samples and dilution water blanks must not exceed the dissolved oxygen saturation value of 9.1 mg/L at 20 Deg. C.

5. "Top off" the sample in the bottle with dilution water being careful not to overfill the bottle. Insert a glass stopper into the neck of the bottle and place a plastic cap on the bottle to keep the water seal from evaporating. Place the BOD samples in the BOD incubator.

6. After 5 days, record the final (Day 5) DO readings of all of the samples and record them on the corresponding work sheet.

Glucose-glutamic acid:

Because the BOD test depends on bacterial activity for valid results, the test can be influenced by the presence of toxins or poor seed material. Periodically, the dilution water quality, seed effectiveness and analytical technique should be checked by making BOD measurements on pure organic compounds and samples with known additions. The standard check solution with a BOD5 of 198.0 mg/L $\pm$ 30.5 mg/L, is a mixture of 150 mg of glucose and 150 mg of glutamic acid brought up to 1L with distilled deionized water in a volumetric flask. Place the solution on a stir plate, insert a magnetic stir bar, and stir for 4-5 minutes. The typical volume utilized for the glucose-glutamic acid (GGA) check sample can range from 4 to 6 mLs per BOD bottle. In terms of percent dilution, a 2% dilution is typically used. As an alternative, if the particular wastewater being analyzed has an identifiable major constituent that contributes to the BOD of the sample, then this compound can be used in place of the GGA mixture.

Temperature Monitoring:

The temperature of the incubator should be monitored at least once per day during normal working hours, whenever compliance monitoring samples are being stored in the incubator or water bath.

The thermometers employed for temperature monitoring of the incubator should be located within the incubator and immersed in water or in saturated air above a volume of water in a sealed container. This way, the opening of the incubator door will not result in rapid changes in the thermometer's reading. Thermometers should be calibrated annually against a National Bureau of Standards traceable thermometer and then documented as to date, time and persons performing the calibration.

Valid Results:

Dilution water blank dissolved oxygen depletions greater than (>) 0.2 mg/L not only invalidate the entire BOD run for reporting purposes, but also require a careful check to find the reason for the high oxygen demand. The most common causes are dirty equipment and/or reagent contamination due to bacteria, algae and mold or contaminated compressed air used to aerate the dilution water.

Although BOD samples are considered invalid when either an inadequate amount of oxygen is consumed or the oxygen is depleted, the BOD of the sample may be reported on the DEP 49 form as estimates when no dilutions for a sample exist that fall within the valid range for oxygen consumption. If an estimated BOD is reported, an explanatory letter detailing the corrective action to be taken should accompany the DEP report.

BOD samples that have DO *depletions* of less than (<) 2 mg/L dissolved oxygen after the five day incubation period will not produce reliable BOD results and are invalid. Larger sample sizes should be used for future BOD runs. Calculate the BOD5 using the largest sample volume and report results as "less than" (<) the calculated value.

BOD samples that have DO c*oncentrations* of less than (<) 1.0 mg/L after the five day incubation period must be considered "depleted" and are invalid. Calculate the BOD5 using the smallest sample volume and report the result as "greater than" (>) the calculated value.

Troubleshooting:

See the above sections on Interferences, Cleaning, Dilution Water, Set-Up, Seeding and Valid Results.

Calculation when sample is not seeded:

BOD5, mg/L =

$$\frac{(\text{Initial DO} - \text{Final DO}) \times 300}{\text{sample volume in mLs}}$$

or,

BOD5, mg/L =

DO depletion X Dilution Factor

where DO Depletion = (Initial DO – Final DO)
and Dilution Factor = 300 divided by Sample Volume in mLs

Calculation of the seed correction factor:

The formula for calculating the seed correction factor is:

Seeded BOD5, mg/L when seed is added directly to each bottle =

Note: * means "multiplied by"

{(DO Depletion) – (Seed Depletion)* f} * Dilution Factor

where DO Depletion = Initial DO – Final DO and
Seed Depletion = Initial Seed DO – Final Seed DO

and $f = \frac{\text{volume of seed in the sample}}{\text{volume of seed in seed control}}$

and Dilution Factor = 300 divided by Sample Volume in mLs

BOD5 calculations from *Standard Methods*

When sample is not seeded:

$$\text{BOD5, mg/L} = \frac{\text{(DO initial – DO final)}}{P}$$

where P = the decimal volumetric fraction of sample used

When sample is seeded:

$$\text{BOD5, mg/L} = \frac{\{\text{(DO depletion) – (Seed Depletion)} * f\}}{P}$$

where DO Depletion = (DO initial – DO final)

Seed Depletion = (Seed Control initial DO – Seed Control final DO)

$f = \frac{\text{volume of seed in the sample}}{\text{volume of seed in seed control}}$

P = the decimal volumetric fraction of sample used.

The above calculation for seeded BOD's is valid only when the seed is added directly to each BOD bottle. If seed is added directly to the dilution water then the seed correction for each dilution used needs to be calculated.

Precision and Accuracy:

The acceptable range for glucose-glutamic acid checks should be 198 ± 30.5 mg/L. Analysts can use 198 mg/L + 30.5 mg/L as the upper control limit and 198

mg/L – 30.5 mg/L as the lower control limit. Alternatively, it is recommended that initially each laboratory perform a minimum of 25 glucose-glutamic acid checks over a period of several weeks or months and calculate the mean and the standard deviation. Once the control limits for this test are completed, then each laboratory should perform a minimum of 1 glucose-glutamic acid check per every ten samples.

With regard to precision, ten percent duplicate or replicate samples should be run. This would result in one duplicate sample or one replicate sample being run every ten samples.

A **Replicate Sample** is defined as a sample that comes from the same bottle and is split in the laboratory and each split sample is analyzed at the same time.

A **Duplicate Sample** is defined as a sample that is taken at the same time but collected in the field in a different bottle. One example of how duplicate samples can be reasonably collected is by and filling two individual bottles at the same time.

*-This procedure has been excerpted in its entirety from *Standard Methods* 18th edition, 1992, pp5-1 to 5-6 and is meant only to summarize and not to be a replacement for *Standard Methods*.

CAUSES OF ERRATIC DISSOLVED OXYGEN READINGS

USING YSI PROBES

YSI Self Stirring BOD Probes

1. Inadequate warm-up time for meter and probe.
2. Wrinkled, loose, or fouled membranes.
3. Large bubbles (> 1/8 inch) in the electrolyte reservoir.
4. Expired electrolyte (KCl).
5. Using a bad batch of the preformed YSI membranes.
6. Sensor Body O-Ring is worn or loose.
7. Gold cathode is tarnished or plated with silver.
8. Silver anode is contaminated.

9. Interference from the presence of any of the following gasses: H_2S, SO_2, Halogens, Neon, or CO.

10. Membrane becomes coated with either oxygen consuming or oxygen producing microorganisms.

11. Heavy residue coats the membrane.

12. Substances that may attack the probe materials such as acids, caustics and strong solvents.

13. Storing the probe in a dry environment.

14. Failure to calibrate the dissolved oxygen meter based upon atmospheric pressure.

Table 1.

Range of Expected BOD Values Based on Sample Size

Sample Volume (mLs)	Dilution Factor	BOD Range (mg/L)	
		minimum	maximum**
1	300	600	2,100
2	150	300	1,050
3	100	200	700
4	75	150	525
5	60	120	420
6	50	100	350
10	30	60	210
15	20	40	140
20	15	30	105
25	12	24	84
30	10	20	70
50	6	12	42
60	5	10	35
75	4	8	28
100	3	6	21
150	2	4	14
300	1	2***	7

** - BOD calculated using an initial DO of 8.0 mg/L. Initial DO values > than 8.0 mg/L to a maximum of 9.1 mg/L will enable the analyst to measure higher BOD values.

*** - Minimum detection level of the BOD test.

Name of Facility
Laboratory SOP

Escherichia coli by Membrane Filter Standard Methods 9213-D

Accepted by MWWCA Lab Committee Date May 12, 2004

Revised by _____ Date _____

Revised by _____ Date _____

Revised by _____ Date _____

Escherichia coli Membrane Filter Procedure

Sample Type: Grab

Container:

Sterilized Screw-Cap Wide-Mouth Glass or Plastic (e.g., Polypropylene) Bottles or Pre-Sterilized Plastic, (e.g., Whirl-Pak) bags. The bottles are sterilized by autoclaving at 15 psi, 121 ^{o}C for 15 minutes. Whirl-Pak bags are shipped sterile from the manufacturer.

If the sample has been chlorinated, use sample containers that contain 1 ml of 1 % sodium thiosulfate (solution prepared every 3 months) for every 100 ml of sample to be collected. The sodium thiosulfate should be placed in containers prior to sterilization. As an alternative, Whirl-Pak bags containing a pre-measured pellet of sodium thiosulfate can be utilized. If samples are taken after dechlorination, the addition of sodium thiosulfate is unnecessary, provided the dechlorinated sample has been tested for the presence of total residual chlorine at the time of sampling and the chlorine concentration is below the detection limit.

When collecting the bacterial samples, it is very important that the sample containers be filled a maximum of 3/4 of the way full. This allows room for thorough shaking and mixing of the sample contents prior to filtration. By following this step, the accuracy of this analysis will be improved.

Preservative:

It is preferable to analyze samples immediately. When bacteriological samples cannot be analyzed within 15 minutes of being sampled, they must be kept in the dark and iced or refrigerated at a temperature of 1-4 ^{o}C during transit to the laboratory. The maximum holding time cannot exceed 6 hours between sample collection and filtration of the sample. Any extension of the holding time, such as shipping the sample to a contract lab, should be approved by the regulatory authority.

Interferences:

Glassware and plasticware that contain substances that inhibit bacterial growth.

Samples that contain residual chlorine must be dechlorinated at the time of collection to prevent inhibition of bacterial growth.

Heavy metals such as copper, lead, zinc, etc. at concentrations > 0.01 mg/L will exert an inhibitory effect on bacterial growth. To eliminate the toxic effect of heavy

metals a chelating agent such as EDTA must be added. As with the dechlorinating agent, the EDTA must be added to the sample container prior to sterilization.

Water samples that contain either colloidal or suspended particulate matter can clog the membrane filter and prevent filtration. In addition, the suspended or colloidal material can cause the bacterial colonies to spread and this may interfere with the identification of the E. coli colonies.

Equipment:

Hot Air Incubator - must maintain a uniform and constant temperature of 35 ^{o}C, and must not vary more than ± 0.5 ^{o}C. Check and record the temperature of the incubator at least once each day of use.

Water Bath Incubator - must maintain a uniform and constant temperature of 44.5 ^{o}C, and must not vary more than ± 0.2 ^{o}C. As an alternative, an accurate solid heat sink incubator is acceptable. Check and record the temperature of the incubator at least once each day of use.

Culture Dishes - use tight-fitting plastic dishes because the membrane filter cultures are submerged in a water bath during incubation. Enclose groups of E. coli cultures in plastic bags or seal individual dishes with waterproof tape to prevent leakage during submersion.

Filter Membranes - should be sterilized, gridded, have a pore size of 0.45 um, and a diameter of 47 mm. An acceptable filter membrane is the Gelman, GN-6 membrane.

Autoclave - capable of maintaining a temperature of 121 ^{o}C and a pressure of 15 psi for at least 60 minutes.

Vacuum Pump - electrical or hand operated that will allow for filtration of samples quickly through a membrane filter.

Filtering Manifold - capable of holding filtration apparatus.

Bunsen Burner or Alcohol Burner - used to flame pipettes, openings of sterile dilution water containers and forceps.

Forceps - capable of handling membrane filters without damage.

Ethyl Alcohol (95% or Denatured) - used as a temporary storage solution while forceps are in use. Alcohol is flamed off before membrane filters are handled.

Rinse Water Bottles - small mouth 500 ml to 1 liter Pyrex bottles used to store sterile rinse water.

Pipettes - sterilized 1 ml and 10 ml capacity. It is recommended that wide-bore milk dilution pipettes be used.

pH Meter - capable of reading to 0.01 pH units and having a combination flat surface pH probe.

Stirrer Hot Plate - used for preparing media.

Reagents:

Stock Phosphate Buffer: Dissolve 34.0 grams of potassium dihydrogen phosphate, (KH_2PO_4), into 500 ml of distilled water and adjust the pH to 7.2 $\pm$ 0.5 with sodium hydroxide, (NaOH), and dilute to 1 liter. Autoclave at 121 ^{o}C, 15 psi for 15 minutes.

Stock Magnesium Chloride Solution: Dissolve 81.1 grams of magnesium chloride, ($MgCl_2.6H_2O$), into 1 liter of distilled water. Autoclave at 121 ^{o}C, 15 psi for 15 minutes.

Buffered Dilution Water: Add 1.25 ml of stock phosphate buffer and 5.0 ml of the stock magnesium chloride solution to 1 liter of distilled water.
Alternatively, you can purchase a phosphate buffer and magnesium chloride concentrate (Hach 21431-66) and follow the directions for preparing the dilution water.

For use in the dilution of bacterial samples, dispense 99 ml $\pm$ 2.0 ml into milk dilution bottles, tighten the screw caps loosely and autoclave for 15 minutes at 121 ^{o}C and a pressure of 15 psi.

When using the buffered dilution water as a rinse water, dispense it into 500 ml to 1 liter Erlenmeyer Flasks or autoclavable plastic wash bottles. When using screw cap bottles, tighten cap loosely. If using either Erlenmeyer Flasks or stoppered bottles, use gauze-wrapped cotton plugs and/or inverted Griffin beakers to prevent contamination. Autoclave for 30 minutes at 121 ^{o}C and a pressure of 15 psi. **(Caution: To avoid the danger of bottles of super-heated water exploding, bottles of sterile dilution water must be allowed to cool to room temperature for 24 hours before being opened.)**

Medium:

m-TEC Medium - The need for uniformity dictates the use of dehydrated media. Never prepare media from basic ingredients when suitable dehydrated media are available. Follow manufacturer's directions for rehydration. To prepare 500 ml, (makes approximately 100 plates), dissolve 22.65 g m TEC agar media in 500 ml of deionized water. Heat with stirrer on hot plate to dissolve completely. Sterilize in the autoclave at 121 ^{o}C, 15 psi for 15 minutes. Dispense approximately 4 to 5 ml of the hot media into sterile 9*50 mm petri dishes. When media has cooled and

solidified, store upside down in a closed plastic bag within a closed box in a refrigerator. Use 1 plate to test pH and record. The pH should be between 7.1 and 7.5. Plates may be stored in this manner for up to 3 months. Commercially available plates may be used if known to give equivalent results.

Urea Substrate - Add 2.0 g Urea, 0.1 g Phenol Red to 100 ml distilled water. Stir on a magnetic stirrer until urea and phenol red are dissolved. Test pH and adjust to between 3.0 and 4.0 with 0.2 N sodium hydroxide (NaOH) or 1 N hydrochloric acid (HCl). The urea substrate should be straw colored. Below a pH of 4.5, the substrate will be yellow, and above a pH of 5.5, it will be red. This substrate should be stored in a refrigerator at 5 ^{o}C and discarded after 1 week. Larger quantities can be frozen and then thawed for use.

Procedure:

1. Use sterile filtration units at the beginning of each filtration series as a minimum precaution to avoid accidental contamination. A filtration series is considered to be interrupted when an interval of 30 minutes or longer elapses between sample filtrations. After such interruption, treat any further sample filtration as a new filtration series and sterilize all membrane filter holders in use.

2. Decontaminate this equipment between successive filtrations by using an ultraviolet sterilizer, flowing steam, or boiling water. In the UV sterilization procedure, a 2 minute exposure is sufficient. Do not expose membrane filter culture preparations to random UV radiation leaks that might emanate from the sterilization cabinet. Use eye protection against stray radiation from any UV sterilizer that is not light tight while in operation.

3. For quality control purposes, it is recommended that a 100 ml Blank Dilution Water sample be run at the beginning and end of each daily sample run. In this way the analyst can verify that the filtration glassware was free of bacterial contamination at the start and end of the sample run.

4. Select the volume of water to be filtered that will yield between 20 and 80 E. coli colonies per membrane. If there is doubt as to the correct dilution to use, then select additional quantities that represent one-tenth and ten times the selected volume.

5. Shake sample bottle vigorously (about 25 times in 7 seconds) to evenly distribute the bacteria. Take care to secure the screw cap or Whirl-Pak bag and prevent leakage during shaking. This procedure is repeated whenever bacteria laden water is being transferred.

6. Sample volumes should be filtered in the order of smallest to largest sample volumes. This will minimize the potential for erroneously high results and help to improve overall test precision.

7. When measuring sample portions less than 50 mL use a sterile wide-bore pipet for initial and subsequent transfers from each container. If pipet becomes contaminated before transfers are completed, replace with a sterile pipet. Use a separate sterile pipet for transfers from each different dilution. Use caution when removing sterile pipettes from the container. To avoid contamination, do not drag the pipet tip across exposed ends of pipettes in the pipet container or across lips and necks of dilution bottles. When removing sample, do not insert pipettes more than 2.5 cm below the surface of the sample or dilution.

8. When discharging sample portions, hold pipet at a 45 ^{o} angle with the tip touching the inside neck of the dilution bottle or filtration apparatus. Lift cap just high enough to insert pipet. If pipet is not a blowout type touch the tip of the pipet against a dry part of the dilution bottle neck.

9. When less than 20 ml of sample (diluted or undiluted) is to be filtered, add approximately 10 ml of sterile dilution water to the funnel before filtration. This increase in water volume aids in making the dispersion of the bacterial suspension more uniform.

10. When measuring samples portions 50 mL or larger, pour directly from the sample container into the filter funnel. Initially, the funnel must be calibrated by measuring volumes of water such as 50 mL and 100 mL in a graduated cylinder, pouring the water into a funnel and then marking the level on the funnel. This must be done even if the manufacturer graduates the funnels.

11. Using sterile forceps, place a sterile membrane filter (grid side up), over a porous plate receptacle. Carefully place matched funnel unit over receptacle and lock it in place. Filter sample under partial vacuum. With filter still in place rinse funnel by filtering three 20 to 30 ml portions of sterile dilution water. Upon completion of final rinse and filtration process disengage vacuum, unlock and remove funnel, immediately remove membrane filter with sterile forceps, and place it on selected medium with a rolling motion to avoid entrapment of air. Insert a sterile rinse water sample (100 ml) after filtration of a series of 10 samples to check for possible cross-contamination or contaminated rinse water.

12. Place the prepared cultures upside down first in the hot air oven at 35 ^{o}C ± 0.5 ^{o}C for 2 hours. Transfer to the water bath after placing the petri dishes in waterproof plastic bags. Submerge these petri dishes upside down in a water bath and incubate for 22 ± 2 hours at 44.5 ± 0.2 ^{o}C. Anchor the dishes below the water surface to maintain critical temperature requirements.

13. After the incubation period in the water bath is completed, check all plates for growth and record on the data sheet those plates without growth. Next, arrange the plates on the lab bench, remove the petri dish covers and lay the label side down on the bench beside the bottom of each petri dish. Place a cellulose absorbent pad in each cover and add between 1.8 to 2 ml of urea substrate to each pad. Enough urea substrate should be added to saturate each pad but not leave standing liquid substrate.

14. Using forceps, carefully lift each membrane containing bacterial colonies and place it without entraining air bubbles on the saturated pad. After 10 to 20 minutes, count the positive colonies with the aid of a lighted magnifying glass or stereomicroscope.

Counting:

Colonies produced by E. coli bacteria on m-TEC medium are various shades of yellow. Negative colonies will be mostly bright blue or purple and some may be gray.

Count the yellow E. coli colonies following a preset plan such as shown in Figure 1. Some colonies may be in contact with the grid lines. In order to reduce error in counting those colonies, a suggested procedure is shown in Figure 2. The colonies would be counted in the squares indicated by the arrows.

All yellow colonies should be counted individually, even if they are in contact with each other. There is a difference between two or more colonies that have grown into contact with each other and single, irregularly shaped colonies that sometimes develop on membrane filters. The single irregular colonies are usually associated with a fiber or particulate matter and these colonies conform to the shape and size of the fiber or particulate. Colonies that have grown together almost always show a very fine line of contact.

Calculation:

Record the number of yellow colonies on only plates that contain between 20 to 80 E. coli colonies. Report the E. coli density as # E. coli/100 ml.

$$\text{E. coli/100ml} = \frac{\text{E. coli colonies counted} * 100}{\text{sample volume (ml)}}$$

If there is more than 1 replicate plate that has between 20 to 80 E. coli colonies, then count each plate separately and calculate the average count for that dilution then perform the E. coli/100 ml calculation.

If there is more than 1 dilution of a sample that has between 20 to 80 E. coli colonies, then count each plate separately and perform the E. coli/100 ml calculation. Next using the formula for arithmetic mean, calculate the average E. coli/100 ml concentration for the sample.

If all the plates contain less than 20 E. coli colonies/, then select that plate that is closest to having 20 E. coli colonies/. Next, calculate the E.coli/100 ml on that plate and report the number as an estimated count.

If none of the plates contain any E. coli colonies, then calculate the E. coli concentration using the largest sample volume filtered. Instead of a zero, use the

number 1 for the E. coli colony count and report the E. coli/100 ml value using the less than, (<), sign in front of the number calculated.

If all of the plates are above the upper limit of 80 E. coli colonies, then select the smallest sample volume and calculate the E. coli/100 ml on that plate using a count of 80. Report the number as being greater than the value calculated by using the greater than, (>), sign.

A geometric mean of bacteria results is often required for regulatory purposes. A geometric mean is calculated by multiplying the results together and then taking the nth root. For example, to find the geometric mean of 40, 2, and 80, first multiply 40*2*80=6400. Because there are three values then take the third (cube) root of 6400, which is 18.6. A geometric mean function is available on most spreadsheet programs.

Disposal:

After the plates are counted for E. coli, the used petri dishes should be placed in an autoclavable bag, autoclaved for thirty minutes at a temperature of 121 ^{o}C and a pressure of 15 p.s.i., and then discarded. Alternatively the plates can be soaked in bleach, rinsed, and then discarded.

Troubleshooting:

Verify that each piece of equipment utilized, (especially the incubator and water bath), meets the user's needs for precision and minimization of bias.

Verify that water used to make reagents, media and dilution water is of a quality suitable for bacteriological analysis. If commercial media and disposable petri dishes are utilized, then testing these materials for bacterial growth inhibition is unnecessary.

Minimize contamination of samples and dilutions through the use of proper aseptic techniques.

Quality Control:

Incubators - Whenever the water bath or hot air incubator is in use, the temperature should be recorded daily. In addition, if a cooler or refrigerator is being used for sample storage, its temperature should be recorded on a daily basis, whenever it is in use.

Media - Document the date, time and pH of every media preparation.

Thermometers – Thermometers should be calibrated annually against a National Bureau of Standards traceable thermometer and then documented as to date, time and persons performing the calibration.

Autoclave - Whenever the autoclave is utilized for bacterial related work, some method to verify that the internal temperature and pressure of 121 ^{o}C and 15 psi respectively have been met. Autoclavable tape or tags are acceptable. An alternative method is to autoclave a glass bottle containing wastewater that has not been disinfected. After cooling, 1/2 ml of the autoclaved wastewater is pipetted into a tube of sterile lauryl tryptose broth and incubated overnight at 35 ^{o}C. If there is no growth in the tube, then sterility has been achieved. Each time the autoclave is used for bacterial related work, staff should document the date, time, purpose (e.g., bottles, media prep), analyst and whether sterility was achieved.

Reagent water - A sample of reagent water should be checked monthly for chlorine residual, conductivity, bacterial growth and this information recorded. In addition, a sample of the deionized water should be sent to the Maine DHS Public Health Laboratory annually to be checked for bacterial suitability and the results of this test kept on file.

UV sterilization unit check – Two 10 mL portions of unchlorinated wastewater are filtered, and the membrane filters are placed in petri dishes with fecal or e. coli media. The open dishes are then placed in the UV light drawer. After one cycle under the UV lights, the dishes are closed up and incubated as usual. For comparison, dilutions such as 0.01 mL and 0.1 mL of the wastewater are filtered and incubated as usual. The percent kill for the UV lamps should be at least 99%. If two successive tests fail, replace the lights.

Positive control samples – A positive control sample, such as diluted (1:1000) effluent prior to disinfection, must be run for each new lot of media.

Duplicate counts – At least once per month, two or more analysts should count the colonies on the same membrane from a positive sample. The counts should agree within 10%.

Precision and Accuracy:

With regard to precision, ten percent duplicate and/or replicate samples should be run. The precision of the duplicates and/or replicates should be +/- 30% calculated as the difference as a percentage of the mean.

A **Replicate Sample** is defined as a sample that comes from the same bottle and is split in the laboratory and each split sample is analyzed at the same time.

A **Duplicate Sample** is defined as a sample that is taken at the same time but collected in the field in a different bottle. One example of how duplicate samples can be reasonably collected is by manually filling two individual bottles at that time.

If quality control and precision and accuracy procedures show there are problems, refer to the Troubleshooting section for corrective actions.

* - This procedure has been excerpted in its entirety from three sources. They are: Standard Methods 18th edition, 1992, pp 9-13 to 9-20 and 9-53 to 9-54; the Maine DEP Methodology for monitoring Escherichia coli; and, Microbiological Methods for Monitoring the Environment, Water and Wastes published by the EPA, (EPA-600/8-78-017). This information is meant only to summarize and not be a replacement for these references.

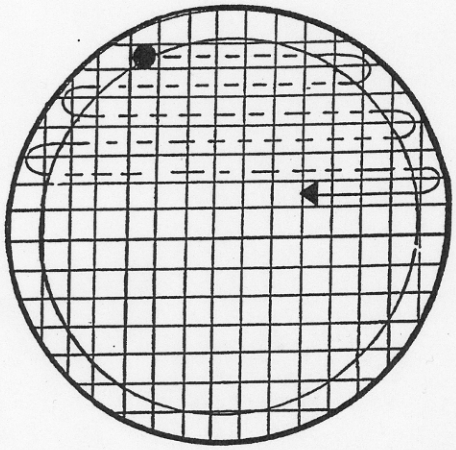


FIGURE I Colony-Counting Pathway. (The inner circle indicates the effective filtering area, dashed line indicates the pathway.)

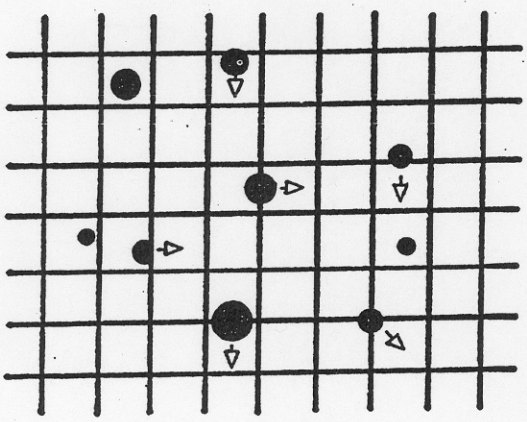


FIGURE II Enlarged Portion of Grid-Marked Square of Filter. (Colonies are counted in squares indicated by the arrow.)

Name of Facility
Laboratory SOP

Escherichia coli by Colisure â Standard Methods 9223 and Idexx

Accepted by MWWCA Lab Committee Date May 12, 2004_

Revised by _____ Date _____

Revised by _____ Date _____

Revised by _____ Date _____

Escherichia coli Colisure â Procedure

Sample Type: Grab

Container:

Idexx 100 mL sample bottles with sodium thiosulfate, or Sterilized Screw-Cap Wide-Mouth Glass or Plastic (e.g., Polypropylene) Bottles or Pre-Sterilized Plastic, (e.g., Whirl-Pak) bags. The Idexx bottles and Whirl-Pak bags are shipped sterile from the manufacturer. The screw-cap bottles are sterilized by autoclaving at 15 psi, 121 ^{o}C for 15 minutes.

If the sample has been chlorinated, use sample containers that contain 1 ml of 1 % sodium thiosulfate (solution prepared every 3 months) for every 100 ml of sample to be collected. The sodium thiosulfate should be placed in containers prior to sterilization. As an alternative, Idexx bottles or Whirl-Pak bags containing a pre-measured amount of sodium thiosulfate can be utilized. If samples are taken after dechlorination, the addition of sodium thiosulfate is unnecessary, provided the dechlorinated sample has been tested for the presence of total residual chlorine at the time of sampling and the chlorine concentration is below the detection limit.

When collecting the bacterial samples, it is very important that the sample containers be filled a maximum of 3/4 of the way full. This allows room for thorough shaking and mixing of the sample contents prior to filtration. By following this step, the accuracy of this analysis will be improved.

Preservative:

It is preferable to analyze samples immediately. When bacteriological samples cannot be analyzed within 15 minutes of being sampled, they must be kept in the dark and iced or refrigerated at a temperature of 1-4 ^{o}C during transit to the laboratory. The maximum holding time cannot exceed 6 hours between sample collection and testing of the sample. Any extension of the holding time, such as shipping the sample to a contract lab, should be approved by the regulatory authority.

Interferences:

Glassware and plasticware that contain substances that inhibit bacterial growth.

Samples that contain residual chlorine must be dechlorinated at the time of collection to prevent inhibition of bacterial growth.

Heavy metals such as copper, lead, zinc, etc. at concentrations > 0.01 mg/L will exert an inhibitory effect on bacterial growth. To eliminate the toxic effect of heavy metals a chelating agent such as EDTA must be added. As with the dechlorinating agent, the EDTA must be added to the sample container prior to sterilization.

Highly colored solutions may interfere with the color change of the Colisure® reaction.

Equipment:

Hot Air Incubator - must maintain a uniform and constant temperature of 35 ^{o}C, and must not vary more than ± 0.5 ^{o}C. Check and record the temperature of the incubator at least once each day of use.

Autoclave - capable of maintaining a temperature of 121 ^{o}C and a pressure of 15 psi for at least 60 minutes.

Idexx Quanti-Tray® Sealer with rubber insert.

Quanti-Trays® – Use Quanti-Tray® for counts from 1-200 and Quanti-Tray®/2000 for counts from 1-2,419.

UV Lamp – Long wave, 365 nm.

Reagents:

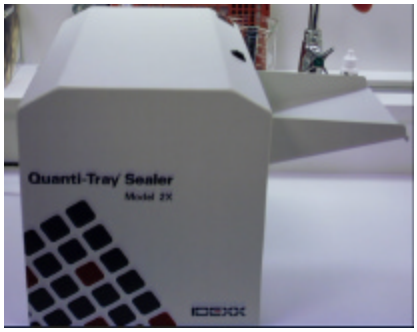
Idexx Antifoam Solution.

Medium:

Idexx Colisure® powder packets.

Procedure:

1. Turn Quanti-Tray® sealing unit on. Unit is ready when green light is lit. Could take up to 10 minutes.



2. If the sample was collected in a wide-mouth bottle or Whirl-Pak bag, shake the sample vigorously (about 25 times in 7 seconds) to evenly distribute the bacteria. Take care to secure the screw cap or Whirl-Pak bag and prevent leakage during shaking. Fill the Idexx plastic bottle with sample up to the 100 mL line.

3. Add Idexx Colisure® powder packet to sample bottle.

4. Add one drop Idexx defoamer to sample bottle and shake until powder dissolves.

5. Pour sample from Idexx 100ml sample bottle into foil pouch. Avoid contact with pouch interior. Maintain the sterile environment.

6. Place Colisure pouch onto rubber insert.

7. Place insert into sealer. Run pouch through small sample squares first.

8. Turn sealer off.

9. Label back of packet with:
    a. Time
    b. Date
    c. Sample Source
    d. Initials

10. Place sealed Idexx sample packet into the incubator (35.0±0.5) for 24 to 48 hrs.

Counting:

After 24 to 48 hours, check cells for purple color. Check purple cells for florescence.

Record number of small and large cells showing purple color and florescence on the data sheet.

Calculation:

Find results on Idexx Quanti-Tray/2000 MPN Table and record data on data sheet.

- If no purple color is observed, the test is negative.
  - Purple indicated presence of total coliform.
  - Purple and florescence indicates presence of E-Coli

- If purple is observed, check for fluorescence by placing the black light within five inches of the sample in a dark environment. Be sure the light is facing away from your eyes and toward the sample.

Disposal:

The used trays should be placed in an autoclavable bag, autoclaved for thirty minutes at a temperature of 121 ^{o}C and a pressure of 15 p.s.i., and then discarded.

Troubleshooting:

Verify that each piece of equipment utilized, (especially the incubator), meets the user's needs for precision and minimization of bias.

Verify that media used is not beyond its expiration date.

Minimize contamination of samples and dilutions through the use of proper aseptic techniques.

Quality Control:

Incubators - Whenever the hot air incubator is in use, the temperature should be recorded daily. In addition, if a cooler or refrigerator is being used for sample storage, its temperature should be recorded on a daily basis, whenever it is in use.

Thermometers - Thermometers should be calibrated annually against a National Bureau of Standards traceable thermometer and then documented as to date, time and persons performing the calibration.

Autoclave - Whenever the autoclave is utilized for bacterial related work, some method to verify that the internal temperature and pressure of 121 ^{o}C and 15 psi respectively have been met. Autoclavable tape or tags are acceptable. An alternative method is to autoclave a glass bottle containing wastewater that has not been disinfected. After cooling, 1/2 ml of the autoclaved wastewater is pipetted into a tube of sterile lauryl tryptose broth and incubated overnight at 35 ^{o}C. If there is no growth in the tube, then sterility has been achieved. Each time the autoclave is

used for bacterial related work, staff should document the date, time, purpose (e.g., bottles, media prep), analyst and whether sterility was achieved.

Positive control samples – A positive control sample, such as diluted (1:1000) effluent prior to disinfection, must be run for each new lot of media. Alternatively, the Idexx Quanti-Cult® QC Kit can be used.

Duplicate counts – At least once per month, two or more analysts should count the purple and fluorescent cells on the same tray from a positive sample. The counts should agree within 10%.

* - This procedure has been excerpted in its entirety from three sources. They are: Standard Methods 18th edition, 1992, pp 9-13 to 9-20; Microbiological Methods for Monitoring the Environment, Water and Wastes published by the EPA, (EPA-600/8-78-017), and the manufacturer's procedures found at www.idexx.com. This information is meant only to summarize and not be a replacement for these references.

Name of Facility
Laboratory SOP

Fecal Coliform by Membrane Filter
Standard Methods 9222-D

Accepted by MWWCA Lab Committee Date May 12, 2004_

Revised by _____ Date _____

Revised by _____ Date _____

Revised by _____ Date _____

*Fecal Coliform Membrane Filter Procedure Std. Method # 9222-D**

Sample Type: Grab

Container:

Sterilized Screw-Cap Wide-Mouth Glass or Plastic (e.g., Polypropylene) Bottles or Pre-Sterilized Plastic, (e.g., Whirl-Pak) bags. The bottles are sterilized by autoclaving at 15 psi, 121 ^{o}C for 15 minutes. Whirl-Pak bags are shipped sterile from the manufacturer.

If the sample has been chlorinated, use sample containers that contain 1 ml of 1 % sodium thiosulfate (solution prepared every 3 months) for every 100 ml of sample to be collected. The sodium thiosulfate should be placed in containers prior to sterilization. As an alternative, Whirl-Pak bags containing a pre-measured pellet of sodium thiosulfate can be utilized. If samples are taken after dechlorination, the addition of sodium thiosulfate is unnecessary, provided the dechlorinated sample has been tested for the presence of total residual chlorine at the time of sampling and the chlorine concentration is below the detection limit.

When collecting the bacterial samples, it is very important that the sample containers be filled a maximum of 3/4 of the way full. This allows room for thorough shaking and mixing of the sample contents prior to filtration. By following this step, the accuracy of this analysis will be improved.

Preservative:

It is preferable to analyze samples immediately. When bacteriological samples cannot be analyzed within 15 minutes of being sampled, they must be kept in the dark and iced or refrigerated at a temperature of 1-4 ^{o}C during transit to the laboratory. The maximum holding time cannot exceed 6 hours between sample collection and filtration of the sample. Any extension of the holding time, such as shipping the sample to a contract lab, should be approved by the regulatory authority.

Interferences:

Glassware and plasticware that contain substances that inhibit bacterial growth.

Samples that contain residual chlorine must be dechlorinated at the time of collection to prevent inhibition of bacterial growth.

Heavy metals such as copper, lead, zinc, etc. at concentrations > 0.01 mg/L will exert an inhibitory effect on bacterial growth. To eliminate the toxic effect of heavy metals a chelating agent such as EDTA must be added. As with the dechlorinating agent, the EDTA must be added to the sample container prior to sterilization.

Water samples that contain either colloidal or suspended particulate matter can clog the membrane filter and prevent filtration. In addition, the suspended or colloidal material can cause the bacterial colonies to spread and this may interfere with the identification of the fecal coliform colonies.

Equipment:

Water Bath Incubator - must maintain a uniform and constant temperature of 44.5 ^{o}C, and must not vary more than ± 0.2 ^{o}C. As an alternative, an accurate solid heat sink incubator is acceptable. Check and record the temperature of the incubator at least once each day of use.

Culture Dishes - use tight-fitting plastic dishes because the membrane filter cultures are submerged in a water bath during incubation. Enclose groups of fecal coliform cultures in plastic bags or seal individual dishes with waterproof tape to prevent leakage during submersion.

Filter Membranes - should be sterilized, gridded, have a pore size of 0.45 um, and a diameter of 47 mm. An acceptable filter membrane is the Gelman, GN-6 membrane.

Autoclave - capable of maintaining a temperature of 121 ^{o}C and a pressure of 15 psi for at least 60 minutes.

Vacuum Pump - electrical or hand operated that will allow for filtration of samples quickly through a membrane filter.

Filtering Manifold - capable of holding the filtration apparatus.

Bunsen Burner or Alcohol Burner - used to flame pipettes, openings of sterile dilution water containers and forceps.

Forceps - capable of handling membrane filters without damage.

Ethyl Alcohol (95% or denatured) - used as a temporary storage solution while forceps are in use. Alcohol is flamed off before membrane filters are handled.

Rinse Water Bottles - small mouth 500 ml to 1 liter Pyrex bottles used to store sterile rinse water.

Pipettes - sterilized 1 ml and 10 ml capacity. It is recommended that wide bore milk dilution pipettes be used.

pH Meter – capable of reading to 0.01 pH units and having a combination pH probe.

Stirrer Hot Plate – used for preparing media

Reagents:

Stock Phosphate Buffer: Dissolve 34.0 grams of potassium dihydrogen phosphate, (KH_2PO_4), into 500 ml of distilled water and adjust the pH to 7.2 ± 0.5 with sodium hydroxide, (NaOH), and dilute to 1 liter. Autoclave at 121 ^{o}C, 15 psi for 15 minutes.

Stock Magnesium Chloride Solution: Dissolve 81.1 grams of magnesium chloride, ($MgCl_2.6H_2O$), into 1 liter of distilled water. Autoclave at 121 ^{o}C, 15 psi for 15 minutes.

Buffered Dilution Water: Add 1.25 ml of stock phosphate buffer and 5.0 ml of the stock magnesium chloride solution to 1 liter of distilled water.
Alternatively, you can purchase a phosphate buffer and magnesium chloride concentrate (Hach 214

For use in the dilution of bacterial samples, dispense 99 ml ± 2.0 ml into milk dilution bottles, tighten the screw caps loosely and autoclave for 15 minutes at 121 ^{o}C and a pressure of 15 psi.

When using the buffered dilution water as a rinse water, dispense it into 500 ml to 1 liter Erlenmeyer Flasks or autoclavable plastic wash bottles. When using screw cap bottles, tighten cap loosely. If using either Erlenmeyer Flasks or stoppered bottles, use gauze-wrapped cotton plugs and/or inverted Griffin beakers to prevent contamination. Autoclave for 30 minutes at 121 ^{o}C and a pressure of 15 psi. **(Caution: To avoid the danger of bottles of super-heated water exploding, bottles of sterile dilution water must be allowed to cool to room temperature for 24 hours before being opened.)**

Medium:

M-FC Medium - The need for uniformity dictates the use of dehydrated media. Never prepare media from basic ingredients when suitable dehydrated media are available.
Broth - Follow manufacturer's directions for rehydration. The final pH should be 7.4 +/- 0.2 at 25°C. Adjust with 0.1N hydrochloric acid (HCl) if necessary. Use within 96 hours. Commercially prepared media in liquid form also may be used if known to give equivalent results.

Agar – Rehydrate mFC Agar in a sterile container. Suspend 52 grams in 1 liter distilled or deionized water and boil to dissolve completely. Add 10 mL of a 1% solution of Bacto Rosolic Acid in 0.2N sodium hydroxide (NaOH). Continue heating for 1 minute. DO NOT AUTOCLAVE. The final pH should be 7.4 +/- 0.2 at 25°C. Adjust with 1N HCl if necessary. Pour 5 mL portions into 50x9 mm petri dished. Let

solidify at room temperature and then store plates in a 1°-5°C refrigerator. Use within 2 weeks.

Procedure:

1. If using M-FC broth, place a sterile absorbent pad in each culture dish and pipet approximately 2 ml of M-FC medium to saturate the pad. Carefully remove any excess liquid from the culture dish. No further preparation is needed for M-FC agar plates.

2. Use sterile filtration units at the beginning of each filtration series as a minimum precaution to avoid accidental contamination. A filtration series is considered to be interrupted when an interval of 30 minutes or longer elapses between sample filtrations. After such interruption, treat any further sample filtration as a new filtration series and sterilize all membrane filter holders in use.

3. Decontaminate this equipment between successive filtrations by using an ultraviolet sterilizer, flowing steam, or boiling water. In the UV sterilization procedure, a 2 minute exposure is sufficient. Do not expose membrane filter culture preparations to random UV radiation leaks that might emanate from the sterilization cabinet. Use eye protection against stray radiation from any UV sterilizer that is not light tight while in operation.

4. For quality control purposes, it is recommended that a 100 ml Blank Dilution Water sample be run at the beginning and end of each daily sample run. In this way the analyst can verify that the filtration glassware was free of bacterial contamination at the start and end of the sample run.

5. Select the volume of water to be filtered that will yield between 20 and 60 fecal coliform colonies per membrane. If there is doubt as to the correct dilution to use, then select additional quantities that represent one-tenth and ten times the selected volume.

6. Shake sample bottle vigorously (about 25 times in 7 seconds) to evenly distribute the bacteria. Take care to secure the screw cap or Whirl-Pak bag and prevent leakage during shaking. This procedure is repeated whenever bacteria laden water is being transferred.

7. Sample volumes should be filtered in the order of smallest to largest sample volumes. This will minimize the potential for erroneously high results and help to improve overall test precision.

8. When measuring sample portions less than 50 mL use a sterile wide-bore pipet for initial and subsequent transfers from each container. If pipet becomes contaminated before transfers are completed, replace with a sterile pipet. Use a separate sterile pipet for transfers from each different dilution. Use caution when removing sterile pipettes from the container. To avoid contamination, do not drag

the pipet tip across exposed ends of pipettes in the pipet container or across lips and necks of dilution bottles. When removing sample, do not insert pipettes more than 2.5 cm below the surface of the sample or dilution.

9. When discharging sample portions, hold pipet at a 45 ^{o} angle with the tip touching the inside neck of the dilution bottle or filtration apparatus. Lift cap just high enough to insert pipet. If pipet is not a blowout type touch the tip of the pipet against a dry part of the dilution bottle neck.

10. When less than 20 ml of sample (diluted or undiluted) is to be filtered, add approximately 10 ml of sterile dilution water to the funnel before filtration. This increase in water volume aids in making the dispersion of the bacterial suspension more uniform.

11. When measuring samples portions 50 mL or larger, pour directly from the sample container into the filter funnel. Initially, the funnel must be calibrated by measuring volumes of water such as 50 mL and 100 mL in a graduated cylinder, pouring the water into a funnel and then marking the level on the funnel. This must be done even if the manufacturer graduates the funnels.

12. Using sterile forceps, place a sterile membrane filter (grid side up), over a porous plate receptacle. Carefully place matched funnel unit over receptacle and lock it in place. Filter sample under partial vacuum. With filter still in place rinse funnel by filtering three 20 to 30 ml portions of sterile dilution water. Upon completion of final rinse and filtration process disengage vacuum, unlock and remove funnel, immediately remove membrane filter with sterile forceps, and place it on selected medium with a rolling motion to avoid entrapment of air. Insert a sterile rinse water sample (100 ml) after filtration of a series of 10 samples to check for possible cross-contamination or contaminated rinse water.

13. Place the prepared cultures in waterproof plastic bags or seal the petri dishes, submerge in a water bath and incubate for 24 $\pm$ 2 hours at 44.5 $\pm$ 0.2 ^{o}C. Anchor the dishes below the water surface to maintain critical temperature requirements. Place all prepared cultures in the water bath within 30 minutes after filtration.

14. After the incubation period in the water bath is completed, check all plates for growth and record on the data sheet those plates without growth. Count the positive colonies with the aid of a lighted magnifying glass or stereomicroscope.

Counting:

Colonies produced by fecal coliform bacteria on M-FC medium are various shades of blue. Pale yellow colonies may be atypical E. coli. Non-fecal coliform colonies are gray to cream-colored. Normally, few non-fecal coliform colonies will be observed on M-FC medium because of selective action of the elevated temperature and addition of rosolic acid salt reagent. Count colonies with a low-power (10 to 15 magnifications) binocular wide field dissecting microscope or other optical device.

Count the blue fecal coliform colonies following a preset plan such as shown in Figure 1. Some colonies may be in contact with the grid lines. In order to reduce error in counting those colonies, a suggested procedure is shown in Figure 2. The colonies would be counted in the squares indicated by the arrows.

All blue colonies should be counted individually, even if they are in contact with each other. There is a difference between two or more colonies that have grown into contact with each other and single, irregularly shaped colonies that sometimes develop on membrane filters. The single irregular colonies are usually associated with a fiber or particulate matter and these colonies conform to the shape and size of the fiber or particulate. Colonies that have grown together almost always show a very fine line of contact.

Calculation:

Record the number of blue colonies on only plates that contain between 20 to 60 fecal coliform colonies. Report fecal coliform density as # fecal coliforms/100 ml.

$$\text{Fecal Coliform/100ml} = \frac{\text{fecal coliform colonies counted} * 100}{\text{sample volume (ml)}}$$

If there is more than 1 replicate plate that has between 20 to 60 fecal coliform colonies, then count each plate separately and calculate the average count for that dilution then perform the fecal coliform/100 ml calculation.

If there is more than 1 dilution of a sample that has between 20 to 60 fecal coliform colonies, then count each plate separately and perform the fecal coliform/100 ml calculation. Next using the formula for arithmetic mean, calculate the average fecal coliform/100 ml concentration for the sample.

If all the plates contain less than 20 fecal coliform colonies, then select that plate that is closest to having 20 fecal coliform colonies. Next, calculate the fecal coliform/100 ml on that plate and report the number as an estimated count.

If none of the plates contain any fecal coliform colonies, then calculate the fecal coliform concentration using the largest sample volume filtered. Instead of a zero, use the number 1 for the fecal coliform colony count and report the fecal coliform/100 ml value using the less than, (<), sign in front of the number calculated.

If all of the plates are above the upper limit of 60 fecal coliform colonies, then select the smallest san

A geometric mean of bacteria results is often required for regulatory purposes. A geometric mean is calculated by multiplying the results together and then taking the nth root. For example, to find the geometric mean of 40, 2, and 80, first multiply 40*2*80=6400. Because there are three values then take the third (cube) root of

6400, which is 18.6. A geometric mean function is available on most spreadsheet programs.

Disposal:

After the plates are counted for fecal coliform, the used petri dishes should be placed in an autoclavable bag, autoclaved for thirty minutes at a temperature of 121 ^{o}C and a pressure of 15 p.s.i., and then discarded. . Alternatively the plates can be soaked in bleach, rinsed, and then discarded.

Troubleshooting:

Verify that each piece of equipment utilized, (especially the incubator and water bath), meets the user's needs for precision and minimization of bias.

Verify that water used to make reagents, media and dilution water is of a quality suitable for bacteriological analysis. If commercial media and disposable petri dishes are utilized, then testing these materials for bacterial growth inhibition is unnecessary.

Minimize contamination of samples and dilutions through the use of proper aseptic techniques.

Quality Control:

Incubators - Whenever the water bath is in use, the temperature should be recorded daily. In addition, if a cooler or refrigerator is being used for sample storage, its temperature should be recorded on a daily basis, whenever it is in use.

Media - Document the date, time and pH of every media preparation.

Thermometers - Thermometers should be calibrated annually against a National Bureau of Standards traceable thermometer and then documented as to date, time and persons performing the calibration.

Autoclave - Whenever the autoclave is utilized for bacterial related work, some method to verify that the internal temperature and pressure of 121 ^{o}C and 15 psi respectively have been met. Autoclavable tape or tags are acceptable. An alternative method is to autoclave a glass bottle containing wastewater that has not been disinfected. After cooling, 1/2 ml of the autoclaved wastewater is pipetted into a tube of sterile lauryl tryptose broth and incubated overnight at 35 ^{o}C. If there is no growth in the tube, then sterility has been achieved. Each time the autoclave is used for bacterial related work, staff should document the date, time, analyst and whether sterility was achieved.

Reagent water - A sample of reagent water should be checked monthly for pH, chlorine residual, cc results of this test kept on file.

UV sterilization unit check – Two 10 mL portions of unchlorinated wastewater are filtered, and the membrane filters are placed in petri dishes with fecal media. The open dishes are then placed in the UV light drawer. After one cycle under the UV lights, the dishes are closed up and incubated as usual. For comparison, dilutions such as 0.01 mL and 0.1 mL of the wastewater are filtered and incubated as usual. The percent kill for the UV lamps should be at least 99%. If two successive tests fail, replace the lights.

Positive control samples – A positive control sample, such as diluted (1:1000) effluent prior to disinfection, must be run for each new lot of media.

Duplicate counts – At least once per month, two or more analysts should count the colonies on the same membrane from a positive sample. The counts should agree with 10%.

Precision and Accuracy:

With regard to precision, ten percent duplicate and/or replicate samples should be run. The precision of the duplicates and/or replicates should be +/- 30% calculated as the difference as a percentage of the mean.

A **Replicate Sample** is defined as a sample that comes from the same bottle and is split in the laboratory and each split sample is analyzed at the same time.

A **Duplicate Sample** is defined as a sample that is taken at the same time but collected in the field in a different bottle. One example of how duplicate samples can be reasonably collected is by manually filling two individual bottles at that time.

If quality control and precision and accuracy procedures show there are problems, refer to the Troubleshooting section for corrective actions.

* - This procedure has been excerpted in its entirety from three sources. They are: Standard Methods 18th edition, 1992, pp 9-13 to 9-20, pp9-53 to 9-54 and pp 9-60 to 9-61; the Maine DEP Methodology for monitoring fecal coliform; and, Microbiological Methods for Monitoring the Environment, Water and Wastes published by the EPA, (EPA-600/8-78-017). This information is meant only to summarize and not be a replacement for these references.

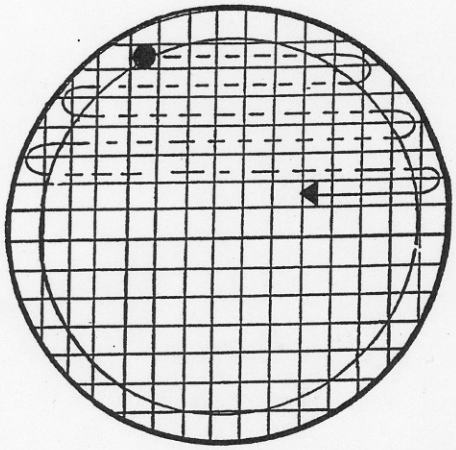


FIGURE I Colony-Counting Pathway. (The inner circle indicates the effective filtering area, dashed line indicates the pathway.)

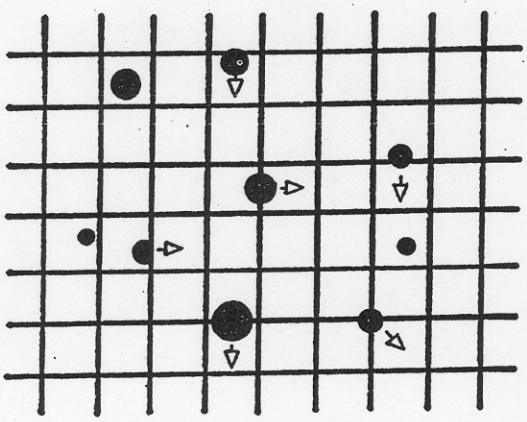


FIGURE II Enlarged Portion of Grid-Marked Square of Filter. (Colonies are counted in squares indicated by the arrow.)